(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 July 2002 (18.07.2002)

PCT

(10) International Publication Number
WO 02/054856 A1

(51) International Patent Classification[7]: A01H 1/06, A61K 31/7076, 31/7088, C12N 1/00, 5/00, C12Q 1/68

(21) International Application Number: PCT/US01/00934

(22) International Filing Date: 15 January 2001 (15.01.2001)

(25) Filing Language: English

(26) Publication Language: English

(71) Applicant *(for all designated States except US)*: **MORPHOTEK INC.** [US/US]; Suite 516, 3624 Market Street, Philadelphia, PA 19104 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **NICOLAIDES, Nicholas, C.** [US/US]; 4 Cider Mill Court, Boothwyn, PA 19061 (US). **GRASSO, Luigi** [US/US]; Apartment #816, 834 Chestnut Street, Philadelphia, PA 19107 (US). **SASS, Philip, M.** [US/US]; 1903 Blackhawk Circle, Audubon, PA 19403 (US).

(74) Agents: **CALDWELL, John, W.** et al.; Woodcock Washburn Kurtz MacKiewicz & Norris LLP, 46th floor, One Liberty Place, Philadelphia, PA 19103 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/054856 A1

(54) Title: CHEMICAL INHIBITORS OF MISMATCH REPAIR

(57) Abstract: Dominant negative alleles of human mismatch repair genes can be used to generate hypermutable cells and organisms. By introducing these genes into cells and transgenic animals, new cell lines and animal varieties with novel and useful properties can be prepared more efficiently than by relying on the natural rate of mutation. Methods of generating mutations in genes of interest and of making various cells mismatch repari defective through the use of chemicals to block mismatch repari in *in vivo* are disclosed.

CHEMICAL INHIBITORS OF MISMATCH REPAIR

TECHNICAL FIELD OF THE INVENTION

The invention is related to the area of mutagenesis. In particular it is related to the field of blocking specific DNA repair processes.

BACKGROUND OF THE INVENTION

Mismatch repair (MMR) is a conserved DNA repair process that is involved in post-replicative repair of mutated DNA sequences that occurs after genome replication. The process involves a group of gene products, including the *mutS* homologs *GTBP, hMSH2,* and *hMSH3* and the *mutL* homologs *hMLH1*, *hPMS1*, and *hPMS2* (Bronner, C.E. *et al.* (1994) *Nature* 368:258-261; Papadopoulos, N. *et al.* (1994) *Science* 263:1625-1629; Leach, F.S. *et al.* (1993) *Cell* 75:1215-1225; Nicolaides, N.C. *et al.* (1994) *Nature* 371:75-80) that work in concert to correct mispaired mono-, di-, and tri-nucleotides, point mutations, and to monitor for correct homologous recombination. Germline mutations in any of the genes involved in this process results in global point mutations, and instability of mono, di and tri-nucleotide repeats (a feature referred to as microsatellite instability (MI)), throughout the genome of the host cell. In man, genetic defects in MMR results in the predisposition to hereditary nonpolyposis colon cancer, a disease in which tumors retain a diploid genome but have widespread MI (Bronner, C.E. *et al.* (1994) *Nature* 368:258-261; Papadopoulos, N. *et al.* (1994) *Science* 263:1625-1629; Leach, F.S. *et al.* (1993) *Cell* 75:1215-1225; Nicolaides, N.C. *et al.* (1994) *Nature* 371:75-80; Harfe B.D., and S. Jinks-Robertson (2000) *An. Rev. Genet.* 34:359-399; Modrich, P. (1994) *Science* 266:1959-1960). Though the mutator defect that arises from MMR deficiency can affect any DNA sequence, microsatellite sequences are particularly sensitive to MMR abnormalities (Peinado, M.A. *et al.*(1992) *Proc. Natl. Acad. Sci. USA* 89:10065-10069). Microsatellite instability is therefore a useful indicator of defective MMR. In addition to its occurrence in virtually all tumors arising in HNPCC patients, MI is found in a small fraction of sporadic tumors with distinctive molecular and phenotypic properties that is due to defective MMR (Perucho, M. (1996) *Biol. Chem.* 377:675-684).

MMR deficiency leads to a wide spectrum of mutations (point mutations, insertions, deletions, recombination, etc.) that can occur throughout the genome of a host

cell. This effect has been found to occur across a diverse array of organisms ranging from but not limited to unicellular microbes, such as bacteria and yeast, to more complex organisms such as *Drosophila* and mammals, including mice and humans (Harfe B.D., and S. Jinks-Robertson (2000) *An. Rev. Genet.* 34:359-399; Modrich, P. (1994) *Science* 266:1959-1960). The ability to block MMR in a normal host cell or organism can result in the generation of genetically altered offspring or sibling cells that have desirable output traits for applications such as but not limited to agriculture, pharmaceutical, chemical manufacturing and specialty goods. A chemical method that can block the MMR process is beneficial for generating genetically altered hosts with commercially valuable output traits. A chemical strategy for blocking MMR *in vivo* offers a great advantage over a recombinant approach for producing genetically altered host organisms. One advantage is that a chemical approach bypasses the need for introducing foreign DNA into a host, resulting in a rapid approach for inactivating MMR and generating genetically diverse offspring or sib cells. Moreover, a chemical process is highly regulated in that once a host organism with a desired output trait is generated, the chemical is removed from the host and its MMR process would be restored, thus fixing the genetic alteration in subsequent generations. The invention described herein is directed to the discovery of small molecules that are capable of blocking MMR, thus resulting in host organisms with MI, a hallmark of MMR deficiency (Peinado, M.A. *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:10065-10069; Perucho, M. (1996) *Biol. Chem.* 377:675-684; Wheeler, J.M. *et al.* (2000) *J. Med. Genet.* 37:588-592; Hoang, J.M. *et al.* (1997) *Cancer Res.* 57:300-303). Moreover, host organisms exhibiting MI are then selected for to identify subtypes with new output traits, such as but not limited to mutant nucleic acid molecules, polypeptides, biochemicals, physical appearance at the microscopic and/or macroscopic level, or phenotypic alterations in a whole organism. In addition, the ability to develop MMR defective host cells by a chemical agent provides a valuable method for creating genetically altered cell hosts for product development. The invention described herein is directed to the creation of genetically altered cell hosts via the blockade of MMR using chemical agents *in vivo*.

The advantages of the present invention are further described in the examples and figures described within this document.

SUMMARY OF THE INVENTION

The invention provides methods for rendering cells hypermutable by blocking MMR activity with chemical agents.

The invention also provides genetically altered cell lines which have mutations introduced through interruption of mismatch repair.

The invention further provides methods to produce an enhanced rate of genetic hypermutation in a cell.

The invention encompasses methods of mutating a gene of interest in a cell, methods of creating cells with new phenotypes, and methods of creating cells with new phenotypes and a stable genome.

The invention also provides methods of creating genetically altered whole organisms and methods of creating whole organisms with new phenotypes.

These and other objects of the invention are provided by one or more of the embodiments described below.

In one embodiment of the invention, a method for screening chemical compounds that block mismatch repair (MMR) is provided. An MMR-sensitive reporter gene containing an out-of-frame polynucleotide repeat in its coding region is introduced into an MMR proficient cell. The cell is grown in the presence of chemicals. Chemicals that alter the genetic structure of the polynucleotide repeat yield a biologically active reporter gene product. Chemicals that disrupt the polynucleotide repeat are identified as MMR blocking agents.

In another embodiment of the invention, an isolated MMR blocking chemical is provided. The chemical can block MMR of a host cell, yielding a cell that exhibits an enhanced rate of hypermutation.

In another embodiment of the invention, a method is provided for introducing a mutation into a gene of interest. A chemical that blocks mismatch repair is added to the culture of a cell line. The cells become hypermutable as a result of the introduction of the chemical. The cell further comprises a gene of interest. The cell is cultured and tested to determine whether the gene of interest harbors a mutation.

In another embodiment of the invention, a method is provided for producing new phenotypes of a cell. A chemical that blocks mismatch repair is added to a cell culture.

The cell becomes hypermutable as a result of the introduction of the chemical. The cell is cultured and tested for the expression of new phenotypes.

In another embodiment of the invention, a method is provided for restoring genetic stability in a cell in which mismatch repair is blocked via a chemical agent. The chemical is removed from the cell culture and the cell restores its genetic stability.

In another embodiment of the invention, a method is provided for restoring genetic stability in a cell with blocked mismatch repair and a newly selected phenotype. The chemical agent is removed from the cell culture and the cell restores its genetic stability and the new phenotype is stable.

In another embodiment of the invention, a chemical method for blocking MMR in plants is provided. The plant is grown in the presence of a chemical agent. The plant is grown and exhibits an enhanced rate of hypermutation.

In another embodiment of the invention, a method for screening chemical inhibitors of MMR in plants *in vivo* is provided. MMR-sensitive plant expression vectors are engineered. The reporter vectors are introduced into plant hosts. The plant is grown in the presence of a chemical agent. The plant is monitored for altered reporter gene function.

In another embodiment of the invention, a method is provided for introducing a mutation into a gene of interest in a plant. A chemical that blocks mismatch repair is added to a plant. The plant becomes hypermutable as a result of the introduction of the chemical. The plant further comprises a gene of interest. The plant is grown. The plant is tested to determine whether the gene of interest harbors a mutation.

In another embodiment of the invention, a method is provided for producing new phenotypes of a plant. A chemical that blocks mismatch repair is added to a plant. The plant becomes hypermutable as a result of the introduction of the chemical. The plant is grown and tested for the expression of new phenotypes.

In another embodiment of the invention, a method is provided for restoring genetic stability in a plant in which mismatch repair is blocked via a chemical agent. The chemical is removed from the plant culture and the plant restores its genetic stability.

In another embodiment of the invention, a method is provided for restoring genetic stability in a plant with blocked mismatch repair and a newly selected phenotype. The

chemical agent is removed from the plant culture and the plant restores its genetic stability and the new phenotype is stable.

These and other embodiments of the invention provide the art with methods that can generate enhanced mutability in microbes, organisms of the protista class, insect cells, mammalian cells, plants, and animals as well as providing cells, plants and animals harboring potentially useful mutations.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows diagrams of mismatch repair (MMR) sensitive reporter genes.

Figure 2 shows a screening method for identifying MMR blocking chemicals.

Figure 3 shows identification of a small chemical that blocks MMR and genetically alters the pCAR-OF vector *in vivo*.

Figure 4 shows shifting of endogenous microsatellites in human cells induced by a chemical inhibitor of MMR.

Figure 5 shows sequence analysis of microsatellites from cells treated with chemical inhibitors of MMR with altered repeats.

Figure 6 shows generation of host organisms with new phenotypes using a chemical blocker of MMR.

Figure 7 shows a schematic diagram of MMR-sensitive reporter gene for plants.

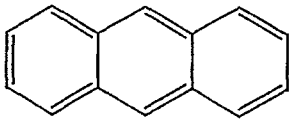
Figure 8 shows derivatives of lead compounds and thereof that are inhibitors of MMR *in vivo*.

DETAILED DESCRIPTION OF THE INVENTION

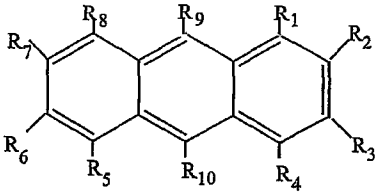
Various definitions are provided herein. Most words and terms have the meaning that would be attributed to those words by one skilled in the art. Words or terms specifically defined herein have the meaning provided in the context of the present invention as a whole and as are typically understood by those skilled in the art. Any conflict between an art-understood definition of a word or term and a definition of the word or term as specifically taught herein shall be resolved in favor of the latter. Headings used herein are for convenience and are not to be construed as limiting.

As used herein the term "anthracene" refers to the compound anthracene. However, when referred to in the general sense, such as "anthracenes," "an anthracene" or "the

anthracene," such terms denote any compound that contains the fused triphenyl core structure of anthracene, i.e.,



regardless of extent of substitution.



In certain preferred embodiments of the invention, the anthracene has the formula:

wherein R_1-R_{10} are independently hydrogen, hydroxyl, amino, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl,O-alkynyl, S-alkynyl, N-alkynyl, aryl, substituted aryl, aryloxy, substituted aryloxy, heteroaryl, substituted heteroaryl, aralkyloxy, arylalkyl, alkylaryl, alkylaryloxy, arylsulfonyl, alkylsulfonyl, alkoxycarbonyl, aryloxycarbonyl, guanidino, carboxy, an alcohol, an amino acid, sulfonate, alkyl sulfonate, CN, NO_2, an aldehyde group, an ester, an ether, a crown ether, a ketone, an organosulfur compound, an organometallic group, a carboxylic acid, an organosilicon or a carbohydrate that optionally contains one or more alkylated hydroxyl groups;

wherein said heteroalkyl, heteroaryl, and substituted heteroaryl contain at least one heteroatom that is oxygen, sulfur, a metal atom, phosphorus, silicon or nitrogen; and

wherein said substituents of said substituted alkyl, substituted alkenyl, substituted alkynyl,

substituted aryl, and substituted heteroaryl are halogen, CN, NO_2, lower alkyl, aryl, heteroaryl, aralkyl, aralkyloxy, guanidino, alkoxycarbonyl, alkoxy, hydroxy, carboxy and amino;

and wherein said amino groups optionally substituted with an acyl group, or 1 to 3 aryl or lower alkyl groups;

or wherein any two of R_1-R_{10} can together form a polyether;

or wherein any two of R_1-R_{10} can, together with the intervening carbon atoms of the anthracene core, form a crown ether.

As used herein, "alkyl" refers to a hydrocarbon containing from 1 to about 20 carbon atoms. Alkyl groups may straight, branched, cyclic, or combinations thereof. Alkyl groups thus include, by way of illustration only, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, cyclopentyl, cyclopentylmethyl, cyclohexyl, cyclohexylmethyl, and the like. Also included within the definition of "alkyl" are fused and/or polycyclic aliphatic cyclic ring systems such as, for example, adamantane. As used herein the term "alkenyl" denotes an alkyl group having at least one carbon-carbon double bond. As used herein the term "alkynyl" denotes an alkyl group having at least one carbon-carbon triple bond.

In some preferred embodiments, the alkyl, alkenyl, alkynyl, aryl, aryloxy, and heteroaryl substituent groups described above may bear one or more further substituent groups; that is, they may be "substituted". In some preferred embodiments these substituent groups can include halogens (for example fluorine, chlorine, bromine and iodine), CN, NO_2, lower alkyl groups, aryl groups, heteroaryl groups, aralkyl groups, aralkyloxy groups, guanidino, alkoxycarbonyl, alkoxy, hydroxy, carboxy and amino groups. In addition, the alkyl and aryl portions of aralkyloxy, arylalkyl, arylsulfonyl, alkylsulfonyl, alkoxycarbonyl, and aryloxycarbonyl groups also can bear such substituent groups. Thus, by way of example only, substituted alkyl groups include, for example, alkyl groups fluoro-, chloro-, bromo- and iodoalkyl groups, aminoalkyl groups, and hydroxyalkyl groups, such as hydroxymethyl, hydroxyethyl, hydroxypropyl, hydroxybutyl, and the like. In some preferred embodiments such hydroxyalkyl groups contain from 1 to about 20 carbons.

As used herein the term "aryl" means a group having 5 to about 20 carbon atoms and which contains at least one aromatic ring, such as phenyl, biphenyl and naphthyl. Preferred aryl groups include unsubstituted or substituted phenyl and naphthyl groups. The term "aryloxy" denotes an aryl group that is bound through an oxygen atom, for example a phenoxy group.

In general, the prefix "hetero" denotes the presence of at least one hetero (i.e., non-carbon) atom, which is in some preferred embodiments independently one to three O, N, S, P, Si or metal atoms. Thus, the term "heteroaryl" denotes an aryl group in which one or more ring carbon atom is replaced by such a heteroatom. Preferred heteroaryl groups include pyridyl, pyrimidyl, pyrrolyl, furyl, thienyl, and imidazolyl groups.

The term "aralkyl" (or "arylalkyl") is intended to denote a group having from 6 to 15 carbons, consisting of an alkyl group that bears an aryl group. Examples of aralkyl groups

In general, the prefix "hetero" denotes the presence of at least one hetero (i.e., non-carbon) atom, which is in some preferred embodiments independently one to three O, N, S, P, Si or metal atoms. Thus, the term "heteroaryl" denotes an aryl group in which one or more ring carbon atom is replaced by such a heteroatom. Preferred heteroaryl groups include pyridyl, pyrimidyl, pyrrolyl, furyl, thienyl, and imidazolyl groups.

The term "aralkyl" (or "arylalkyl") is intended to denote a group having from 6 to 15 carbons, consisting of an alkyl group that bears an aryl group. Examples of aralkyl groups include benzyl, phenethyl, benzhydryl and naphthylmethyl groups.

The term "alkylaryl" (or "alkaryl") is intended to denote a group having from 6 to 15 carbons, consisting of an aryl group that bears an alkyl group. Examples of aralkyl groups include methylphenyl, ethylphenyl and methylnaphthyl groups.

The term "arylsulfonyl" denotes an aryl group attached through a sulfonyl group, for example phenylsulfonyl. The term "alkylsulfonyl" denotes an alkyl group attached through a sulfonyl group, for example methylsulfonyl.

The term "alkoxycarbonyl" denotes a group of formula -C(=O)-O-R where R is alkyl, alkenyl, or alkynyl, where the alkyl, alkenyl, or alkynyl portions thereof can be optionally substituted as described herein.

The term "aryloxycarbonyl" denotes a group of formula -C(=O)-O-R where R is aryl, where the aryl portion thereof can be optionally substituted as described herein.

The terms "arylalkyloxy" or "aralkyloxy" are equivalent, and denote a group of formula -O-R′-R″, where R′ is R is alkyl, alkenyl, or alkynyl which can be optionally substituted as described herein, and wherein R″ denotes a aryl or substituted aryl group.

The terms "alkylaryloxy" or "alkaryloxy" are equivalent, and denote a group of formula -O-R′-R″, where R′ is an aryl or substituted aryl group, and R″ is alkyl, alkenyl, or alkynyl which can be optionally substituted as described herein.

As used herein, the term "aldehyde group" denotes a group that bears a moiety of formula -C(=O)-H. The term "ketone" denotes a moiety containing a group of formula -R-C(=O)-R=, where R and R= are independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, or alkaryl, each of which may be substituted as described herein.

As used herein, the term "ester" denotes a moiety having a group of formula -R-C(=O)-O-R= or -R-O-C(=O)-R= where R and R= are independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, or alkaryl, each of which may be substituted as described herein.

The term "ether" denotes a moiety having a group of formula -R-O-R= or where R and R= are independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, or alkaryl, each of which may be substituted as described herein.

The term "crown ether" has its usual meaning of a cyclic ether containing several oxygen atoms. As used herein the term "organosulfur compound" denotes aliphatic or aromatic sulfur containing compounds, for example thiols and disulfides. The term "organometallic group" denotes an organic molecule containing at least one metal atom.

The term "organosilicon compound" denotes aliphatic or aromatic silicon containing compounds, for example alkyl and aryl silanes.

The term "carboxylic acid" denotes a moiety having a carboxyl group, other than an amino acid.

As used herein, the term "amino acid" denotes a molecule containing both an amino group and a carboxyl group. In some preferred embodiments, the amino acids are α-, β-, γ- or δ-amino acids, including their stereoisomers and racemates. As used herein the term "L-amino acid" denotes an α-amino acid having the L configuration around the α-carbon, that is, a carboxylic acid of general formula $CH(COOH)(NH_2)$-(side chain), having the L-configuration. The term "D-amino acid" similarly denotes a carboxylic acid of general formula $CH(COOH)(NH_2)$-(side chain), having the D-configuration around the α-carbon. Side chains of L-amino acids include naturally occurring and non-naturally occurring moieties. Non-naturally occurring (i.e., unnatural) amino acid side chains are moieties that are used in place of naturally occurring amino acid side chains in, for example, amino acid analogs. *See*, for example, Lehninger, *Biochemistry*, Second Edition, Worth Publishers, Inc, 1975, pages 72-77, incorporated herein by reference. Amino acid substituents may be attached through their carbonyl groups through the oxygen or carbonyl carbon thereof, or through their amino groups, or through functionalities residing on their side chain portions.

As used herein "polynucleotide" refers to a nucleic acid molecule and includes genomic DNA cDNA, RNA, mRNA and the like.

As used herein "antisense oligonucleotide" refers to a nucleic acid molecule that is complementary to at least a portion of a target nucleotide sequence of interest and specifically hybridizes to the target nucleotide sequence under physiological conditions.

As used herein "inhibitor of mismatch repair" refers to an agent that interferes with at least one function of the mismatch repair system of a cell and thereby renders the cell more

susceptible to mutation.

As used herein "hypermutable" refers to a state in which a cell *in vitro* or *in vivo* is made more susceptible to mutation through a loss or impairment of the mismatch repair system.

As used herein "agents," "chemicals," and "inhibitors" when used in connection with inhibition of MMR refers to chemicals, oligonucleotides, analogs of natural substrates, and the like that interfere with normal function of MMR.

Methods for developing hypermutable cells and whole organisms have been discovered by taking advantage of the conserved mismatch repair (MMR) process of a host. Dominant negative alleles of MMR genes, when introduced into cells or transgenic animals, increase the rate of spontaneous mutations by reducing the effectiveness of DNA repair and thereby render the cells or animals hypermutable. Hypermutable microbes, protozoans, insects, mammalian cells, plants or whole animals can then be utilized to develop new mutations in a gene of interest. It has been discovered that chemicals that block MMR, and thereby render cells hypermutable, is an efficient way to introduce mutations in cells and genes of interest. In addition to destabilizing the genome of cells exposed to chemicals that inhibit MMR activity may be done transiently, allowing cells to become hypermutable, and removing the chemical exposure after the desired effect (e.g., a mutation in a gene of interest) is achieved. The chemicals that inhibit MMR activity that are suitable for use in the invention include, but are not limited to, anthracene derivatives, nonhydrolyzable ATP analogs, ATPase inhibitors, antisense oligonucleotides that specifically anneal to polynucleotides encoding mismatch repair proteins, DNA polymerase inhibitors, and exonuclease inhibitors. These chemicals can enhance the rate of mutation due to inactivation of MMR yielding clones or subtypes with altered biochemical properties. Methods for identifying chemical compounds that inhibit MMR *in vivo* are also described herein.

The process of MMR, also called mismatch proofreading, is carried out by a group of protein complexes in cells ranging from bacteria to man (Harfe B.D., and S. Jinks-Robertson (2000) *An. Rev. Genet.* 34:359-399; Modrich, P. (1994) *Science* 266:1959-1960). An MMR gene is a gene that encodes for one of the proteins of such a mismatch repair complex. Although not wanting to be bound by any particular theory of mechanism of action, an MMR complex is believed to detect distortions of the DNA helix

resulting from non-complementary pairing of nucleotide bases. The non-complementary base on the newer DNA strand is excised, and the excised base is replaced with the appropriate base, which is complementary to the older DNA strand. In this way, cells eliminate many mutations that occur as a result of mistakes in DNA replication.

Dominant negative alleles cause an MMR defective phenotype even in the presence of a wild-type allele in the same cell. An example of a dominant negative allele of an MMR gene is the human gene *hPMS2-134* (SEQ ID NO:25), which carries a truncating mutation at codon 134 (Nicolaides, N.C. *et al.* (1998) *Mol. Cell. Biol.* 18:1635-1641). The mutation causes the product of this gene to abnormally terminate at the position of the 134th amino acid, resulting in a shortened polypeptide containing the N-terminal 133 amino acids (SEQ ID NO:24). Such a mutation causes an increase in the rate of mutations, which accumulate in cells after DNA replication. Expression of a dominant negative allele of a mismatch repair gene results in impairment of mismatch repair activity, even in the presence of the wild-type allele.

The MMR process has been shown to be blocked by the use of nonhydrolyzable forms of ATP (Galio, L. *et al.* (1999) *Nucl. Acids Res.* 27:2325-2331; Allen, D.J. *et al.* (1997) *EMBO J.* 16:4467-4476; Bjornson, K.P. *et al.* (2000) *Biochem.* 39:3176-3183). However, it has not been demonstrated that chemicals can block MMR activity in cells. Such chemicals can be identified by screening cells for defective MMR activity. Cells from bacteria, yeast, fungi, insects, plants, animals, and humans can be screened for defective mismatch repair. Genomic DNA, cDNA, or mRNA from any cell can be analyzed for variations from the wild type sequences in cells or organisms grown in the presence of MMR blocking compounds. Various techniques of screening can be used. The suitability of such screening assays, whether natural or artificial, for use in identifying hypermutable cells, insects, fungi, plants or animals can be evaluated by testing the mismatch repair activity caused by a compound or a mixture of compounds, to determine if it is an MMR inhibitor.

A cell, a microbe, or a whole organism such as an insect, fungus, plant or animal in which a chemical inhibitor of mismatch repair has been treated will become hypermutable. This means that the spontaneous mutation rate of such cells or whole organism is elevated compared to cells or animals without such treatment. The degree of elevation of the spontaneous mutation rate can be at least 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, 100-

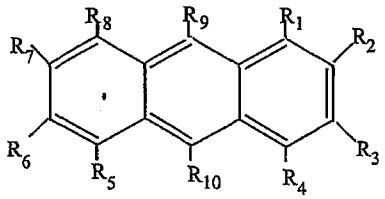
fold, 200-fold, 500-fold, or 1000-fold that of the normal cell or animal. The use of chemical mutagens such as, but limited to, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), methane sulfonate, dimethyl sulfonate, O6-methyl benzadine, ethyl methanesulfonate (EMS), methylnitrosourea (MNU), ethylnitrosourea (ENU), *etc.* can be used in MMR defective cells or whole organisms to increase the rates an additional 10 to 100 fold that of the MMR deficiency itself.

According to one aspect of the invention, a screening assay for identifying chemical inhibitors of MMR is developed and employed. A chemical compound can be in any form or class ranging from but not limited to amino acid, steroidal, aromatic, or lipid precursors. The chemical compound can be naturally occurring or made in the laboratory. The screening assay can be natural such as looking for altered endogenous repeats within an host organism's genome (as demonstrated in Figs. 4 and 5), or made in the laboratory using an MMR-sensitive reporter gene as demonstrated in Figs. 1-3).

The chemical compound can be introduced into the cell by supplementing the growth medium, or by intracellular delivery such as but not limited to using microinjection or carrier compounds.

According to another aspect of the invention, a chemical compound from the anthracene class can be exposed to MMR proficient cells or whole organism hosts, the host is grown and screened for subtypes containing genetically altered genes with new biochemical features.

The anthracene compounds that are suitable for use in the invention include, but are not limited to anthracenes having the formula:



wherein R_1-R_{10} are independently hydrogen, hydroxyl, amino, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl,O-alkynyl, S-alkynyl, N-alkynyl, aryl, substituted aryl, aryloxy, substituted aryloxy, heteroaryl, substituted heteroaryl, aralkyloxy, arylalkyl, alkylaryl, alkylaryloxy, arylsulfonyl, alkylsulfonyl, alkoxycarbonyl, aryloxycarbonyl, guanidino, carboxy, an alcohol,

an amino acid, sulfonate, alkyl sulfonate, CN, NO_2, an aldehyde group, an ester, an ether, a crown ether, a ketone, an organosulfur compound, an organometallic group, a carboxylic acid, an organosilicon or a carbohydrate that optionally contains one or more alkylated hydroxyl groups;

wherein said heteroalkyl, heteroaryl, and substituted heteroaryl contain at least one heteroatom that is oxygen, sulfur, a metal atom, phosphorus, silicon or nitrogen; and

wherein said substituents of said substituted alkyl, substituted alkenyl, substituted alkynyl,

substituted aryl, and substituted heteroaryl are halogen, CN, NO_2, lower alkyl, aryl, heteroaryl, aralkyl, aralkyloxy, guanidino, alkoxycarbonyl, alkoxy, hydroxy, carboxy and amino;

and wherein said amino groups optionally substituted with an acyl group, or 1 to 3 aryl or lower alkyl groups;

or wherein any two of R_1-R_{10} can together form a polyether;

or wherein any two of R_1-R_{10} can, together with the intervening carbon atoms of the anthracene core, form a crown ether.

The method of the invention also encompasses inhibiting MMR with an anthracene of the above formula wherein R_5 and R_6 are hydrogen, and the remaining substituents are as described above.

The some embodiments, in the anthracene compound R_1-R_{10} are independently hydrogen, hydroxyl, alkyl, aryl, arylaklyl, or hydroxyalkyl. In other embodiments, R_1-R_{10} are independently hydrogen, hydroxyl, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, phenyl, tolyl, hydroxymethyl, hydroxypropyl, or hydroxybutyl.

In specific embodiments of the invention the anthracenes include, but are not limited to 1,2-dimethylanthracene, 9,10-dimethyl anthracene, 7,8-dimethylanthracene, 9,10-diphenylanthracene, 9,10-dihydroxymethylanthracene, 9-hydroxymethyl-10-methylanthracene, dimethylanthracene-1,2-diol, 9-hydroxymethyl-10-methylanthracene-1,2-diol, 9-hydroxymethyl-10-methylanthracene-3,4-diol, 9, 10-di-m-tolyanthracene, and the like.

The chiral position of the side chains of the anthracenes is not particularly limited and may be any chiral position and any chiral analog. The anthracenes may also comprise a stereoisomeric forms of the anthracenes and includes any isomeric analog.

Examples of hosts are but not limited to cells or whole organisms from human, primate, mammal, rodent, plant, fish, reptiles, amphibians, insects, fungi, yeast or microbes of prokaryotic origin.

Yet another aspect of the invention is the use of ATP analogs capable of blocking ATPase activity required for MMR. MMR reporter cells are screened with ATP compound libraries to identify those compounds capable of blocking MMR *in vivo*. Examples of ATP analogs that are useful in blocking MMR activity include, but are not limited to, nonhydrolyzable forms of ATP such as AMP-PNP and ATP[gamma]S block the MMR activity (Galio, L. *et al.* (1999) *Nucl. Acids Res.* 27:2325-2331; Allen, D.J. *et al.* (1997) *EMBO J.* 16:4467-4476; Bjornson K.P. *et al.* (2000) *Biochem.* 39:3176-3183).

Yet another aspect of the invention is the use of nuclease inhibitors that are able to block the exonuclease activity of the MMR biochemical pathway. MMR reporter cells are screened with nuclease inhibitor compound libraries to identify compounds capable of blocking MMR *in vivo*. Examples of nuclease inhibitors that are useful in blocking MMR activity include, but are not limited to analogs of N-Ethylmaleimide, an endonuclease inhibitor (Huang, Y.C., et.al. (1995) *Arch. Biochem. Biophys.* 316:485), heterodimeric adenine-chain-acridine compounds, exonulcease III inhibitors (Belmont P, et.al., *Bioorg Med Chem Lett* (2000) 10:293-295), as well as antibiotic compounds such as Heliquinomycin, which have helicase inhibitory activity (Chino, M, et.al. *J. Antibiot. (Tokyo)* (1998) 51:480-486).

Another aspect of the invention is the use of DNA polymerase inhibitors that are able to block the polymerization required for mismatch-mediated repair. MMR reporter cells are screened with DNA polymerase inhibitor compound libraries to identify those compounds capable of blocking MMR *in vivo*. Examples of DNA polymerase inhibitors that are useful in blocking MMR activity include, but are not limited to, analogs of actinomycin D (Martin, S.J., et.al. (1990) *J. Immunol.* 145:1859), Aphidicolin (Kuwakado, K. et.al. (1993) *Biochem. Pharmacol.* 46:1909) 1-(2'-Deoxy-2'-fluoro-beta-L-arabinofuranosyl)-5-methyluracil (L-FMAU) (Kukhanova M, et.al., *Biochem Pharmacol* (1998) 55:1181-1187), and 2',3'-dideoxyribonucleoside 5'-triphosphates (ddNTPs) (Ono, K., et.al., *Biomed Pharmacother* (1984) 38:382-389).

In yet another aspect of the invention, antisense oligonucleotides are administered to cells to disrupt at least one function of the mismatch repair process. The antisense

polynucleotides hybridize to MMR polynucleotides. Both full-length and antisense polynucleotide frgaments are suitable for use. "Antisense polynucleotide fragments" of the invention include, but are not limited to polynuclotides that specifically hybridize to an MMR encoding RNA (as determined by sequence comparison of nucleotides encoding the MMR to nucleotides encoding other known molecules). Identification of sequences that are substantially unique to MMR-encoding polynucleotides can be ascertained by analysis of any publicly available sequence database and/or with any commercially available sequence comparison programs. Antisense molecules may be generated by any means including, but not limited to chemical synthesis, expression in an *in vitro* transcription reaction, through expression in a transformed cell comprising a vector that may be transcribed to produce antisense molecules, through restriction digestion and isolation, through the polymerase chain reaction, and the like.

Antisense oligonucleotides, or fragments thereof may include the nucleotide sequences set forth in SEQ ID NOs:15, 17, 19, 21, 23, 25, 27, and 29 or sequences complementary or homologous thereto, for example. Those of skill in the art recognize that the invention may be predicted using any MMR gene. Specifically, antisense nucleic acid molecules comprise a sequence complementary to at least about 10, 15, 25, 50, 100, 250 or 500 nucleotides or an entire MMR encoding sequence. Preferably, the antisense oligonucleotides comprise a sequence complementary to about 15 consecutive nucleotides of the coding strand of the MMR encoding sequence.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding an MMR protein. The coding strand may also include regulatory regions of the MMR sequence. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues (*e.g.*, the protein coding region of human PMS2 corresponds to the coding region SEQ ID NO:17). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding an MMR protein. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions (UTR)).

Preferably, antisense oligonucleotides are directed to regulatory regions of a nucleotide sequence encoding an MMR protein, or mRNA corresponding thereto,

including, but not limited to, the initiation codon, TATA box, enhancer sequences, and the like. Given the coding strand sequences provided herein, antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of an MMR mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of an MMR mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of an MMR mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length.

Screening is any process whereby a chemical compound is exposed to a cell or whole organism. The process of screening can be carried out using but not limited to a whole animal, plant, insect, microbe, or by using a suspension of one or more isolated cells in culture. The cell can be any type of eukaryotic or prokaryotic cell, including, for example, cells isolated from humans or other primates, mammals or other vertebrates, invertebrates, and single celled organisms such as protozoa, yeast, or bacteria.

In general, screening will be carried out using a suspension of cells, or a single cell, but other methods can also be applied as long as a sufficient fraction of the treated cells or tissue is exposed so that isolated cells can be grown and utilized. Techniques for chemical screening are well known to those in the art. Available techniques for screening include cell-based assays, molecular assays, and whole organism-based assays. Compounds can be added to the screening assays of the invention in order to identify those agents that are capable of blocking MMR in cells.

The screening assays of the invention provide a system wherein a cell, cells or a whole organism is contacted with a candidate compound and then tested to determine whether mismatch repair has been adversely affected. The method in which MMR is analyzed may be any known method, including, but not limited to analysis of the molecular sequence of the MMR gene, and analyzing endogenous repeats in the subject's genome. Further, the invention provides a convenient assay to analyze the effects of candidate agents on reporter genes transfected into cells.

MMR-inhibitors identified by the methods of the invention can be used to generate new mutations in one or more gene(s) of interest. A gene of interest can be any gene naturally possessed by a cell line, microbe or whole organism. An advantage of using

chemicals rather than recombinant technologies to block MMR are that the process is faster; there is no need to produce stable clones with a knocked out MMR gene or a clone expressing a dominant negative MMR gene allele. Another advantage is that host organisms need not be screened for integrated knock out targeting vectors or stable expression of a dominant negative MMR gene allele. Finally, once a cell, plant or animal has been exposed to the MMR-blocking compound and a new output trait is generated, the MMR process can be restored by removal of compound. Mutations can be detected by analyzing the genotype of the cell, or whole organism, for example, by examining the sequence of genomic DNA, cDNA, messenger RNA, or amino acids associated with the gene of interest. Mutations can also be detected by screening for new output traits such as hypoxanthine-guanine phosphoribosyltransferase (HPRT) revertants. A mutant polypeptide can be detected by identifying alterations in electrophoretic mobility, spectroscopic properties, or other physical or structural characteristics of a protein encoded by a mutant gene. One can also screen for altered function of the protein *in situ*, in isolated form, or in model systems. One can screen for alteration of any property of the cell, plant or animal associated with the function of the gene of interest.

Several advantages exist in generating genetic mutations by blocking MMR *in vivo* in contrast to general DNA damaging agents such as MNNG, MNU and EMS. Cells with MMR deficiency have a wide range of mutations dispersed throughout their entire genome in contrast to DNA damaging agents such as MNNG, MNU, EMS and ionizing radiation. Another advantage is that mutant cells that arise from MMR deficiency are diploid in nature and do not lose large segments of chromosomes as is the case of DNA damaging agents such as EMS, MNU, and ionizing radiation (Honma, M. *et al.* (1997) *Mutat. Res.* 374:89-98). This unique feature allows for subtle changes throughout a host's genome that leads to subtle genetic changes yielding genetically stable hosts with commercially important output traits.

The invention also encompasses blocking MMR in vivo and in vitro and further exposing the cells or organisms to a chemical mutagen in order to increase the incidence of genetic mutation.

The invention also encompasses withdrawing exposure to inhibitors of mismatch repair once a desired mutant genotype or phenotype is generated such that the mutations are thereafter maintained in a stable genome.

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples, which are provided herein for purposes of illustration only, and are not intended to limit the scope of the invention.

EXAMPLES

EXAMPLE 1: Generation of a cell-based screening assay to identify chemicals capable of inactivating mismatch repair *in vivo*.

A hallmark of MMR deficiency is the generation of unstable microsatellite repeats in the genome of host cells (Peinado, M.A. *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:10065-10069; Strand, M. *et al.* (1993) *Nature* 365:274-276; Parsons, R. *et al.* (1993) *Cell* 75:1227-1236). This phenotype is referred to as microsatellite instability (MI) (Harfe, B.D. and S. Jinks-Robertson (2000) *Ann. Rev. Genet.* 34:359-399; Modrich, P. (1994) *Science* 266:1959-1960; Peinado, M.A. *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:10065-10069; Perucho, M. (1996) *Biol. Chem.* 377:675-684; Hoang, J.M. *et al.* (1997) *Cancer Res.* 57:300-303; Strand, M. *et al.*(1993) *Nature* 365:274-276). MI consists of deletions and/or insertions within repetitive mono-, di- and/or tri nucleotide repetitive sequences throughout the entire genome of a host cell. Extensive genetic analysis of eukaryotic cells have found that the only biochemical defect that is capable of producing MI is defective MMR (Harfe, B.D. and S. Jinks-Robertson (2000) *Ann. Rev. Genet.* 34:359-399; Modrich, P. (1994) *Science* 266:1959-1960; Peinado, M.A. *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:10065-10069; Perucho, M. (1996) *Biol. Chem.* 377:675-684; Hoang, J.M. *et al.* (1997) *Cancer Res.* 57:300-303; Strand, M. *et al.*(1993) *Nature* 365:274-276). In light of this unique feature that defective MMR has on promoting microsatellite instability, endogenous MI is now used as a biochemical marker to survey for lack of MMR activity within host cells (Hoang, J.M. *et al.* (1997) *Cancer Res.* 57:300-303).

A method used to detect MMR deficiency in eukaryotic cells is to employ a reporter gene that has a polynucleotide repeat inserted within the coding region that disrupts its reading frame due to a frame shift. In the case where MMR is defective, the reporter gene will acquire random mutations (*i.e.*, insertions and/or deletions) within the polynucelotide repeat yielding clones that contain a reporter with an open reading frame. This reporter gene can be of any biochemical pathway such as but not limited to β-glucoronidase, β-galactosidase, neomycin resistant gene, hygromycin resistance gene,

green fluorescent protein, and the like. A schematic diagram of MMR-sensitive reporters are shown in Fig. 1, where the polynucleotide repeat can consist of mono-, di-, tri- or tetra-nucleotides. We have employed the use of a β-galactosidase MMR-sensitive reporter gene to measure for MMR activity in H36 cells, which are a murine hybridoma cell line. The reporter construct used is called pCAR-OF, which contains a hygromycin resistance (HYG) gene plus a β-galactosidase gene with a 29 bp out-of-frame poly-CA tract inserted at the 5' end of its coding region. The pCAR-OF reporter cannot generate β-galactosidase activity unless a frame-restoring mutation (*i.e.*, insertion or deletion) arises following transfection. This line has been shown to be sensitive to inactivated MMR where using a dominant negative MMR gene allele has found this condition to result in the production of β-galactosidase (unpublished data). An example of these data using the dominant negative PMS134 allele is shown in Table 1. Briefly, H36 cells were each transfected with an expression vector containing the PMS134 allele (referred to as HB134) or empty vector and the pCAR-OF vector in duplicate reactions using the protocol below. The PMS134 gene is cloned into the pEF expression vector, which contains the elongation factor promoter upstream of the cloning site followed by a mammalian polyadenylation signal. This vector also contains the NEOr gene that allows for selection of cells in G418 to identify those retaining this plasmid. Briefly, cells were transfected with 1 μg of the PMS134 or empty vector using polyliposomes following the manufacturer's protocol (Life Technologies). Cells were then selected in 0.5 mg/ml of G418 for 10 days and G418 resistant cells were pooled together to analyze for gene expression. PMS134 positive cells, which were determined by RT-PCR and western blot (not shown) were expanded and transfected with the pCAR-OF reporter gene that contains a hygromycin (HYG) resistance gene as reporter using the protocol described above. Cells were selected in 0.5 mg/ml G418 and 0.5mg/ml HYG to select for cells retaining both the MMR effector and the pCAR-OF reporter plasmids. All cultures transfected with the pCAR vector resulted in a similar number of HYG/G418 resistant cells. Cultures were then expanded and tested for β-galactosidase activity *in situ* as well as by biochemical analysis of cell extracts. For *in situ* analysis, 100,000 cells were harvested and fixed in 1% gluteraldehyde, washed in phosphate buffered saline solution and incubated in 1 ml of X-gal substrate solution [0.15 M NaCl, 1 mM $MgCl_2$, 3.3 mM $K_4Fe(CN)_6$, 3.3 mM $K_3Fe(CN)_6$, 0.2% X-Gal] in 24 well plates for 2 hours at 37°C. Reactions were stopped in 500 mM sodium bicarbonate

solution and transferred to microscope slides for analysis. Three fields of 200 cells each were counted for blue (β-galactosidase positive cells) or white (β-galactosidase negative cells) to assess for MMR inactivation. Table 1 shows the results from these studies. While no β-galactosidase positive cells were observed in H36 empty vector cells and 10% of the cells per field were β-galactosidase positive in HB134 cultures.

Table 1. β-galactosidase expression of H36 empty vector and HB134 cells transfected with pCAR-OF reporter vectors. Cells were transfected with the pCAR-OF reporter plasmid. Transfected cells were selected in HYG and G418, expanded and stained with X-gal solution to measure for β-galactosidase activity (blue colored cells). 3 fields of 200 cells each were analyzed by microscopy. The results below represent the mean +/- standard deviation of these experiments.

Table 1.

CELL LINE	# BLUE CELLS
H36 empty vector	0 +/- 0
HB134	20 +/- 3

Cultures can be further analyzed by biochemical assays using cell extracts to measure β-galactosidase activity as previously described (Nicolaides, N.C. *et al.* (1998) *Mol. Cell. Biol.* 18:1635-1641).

The data described in Table 1 show that by inhibiting the MMR activity of an MMR proficient cell host can result in MI and the altering of microsatellites in the pCAR-OF vector results in cells that produce functional β-galactosidase enzyme. The use of the H36pCAR-OF cell line can now be used to screen for chemicals that are able to block MMR of the H36 cell line.

EXAMPLE 2: Screening assays for identifying chemical blockers of MMR.

A method for screening chemical libraries is provided in this example using the H36pCAR-OF cell line described in Example 1. This cell line is a hardy, stable line that can be formatted into 96-well microtiter plates for automated screening for chemicals that

specifically block MMR. An overview of the screening process is given in Figure 2, however, the process is not limited to the specifications within this example. Briefly, 10,000 cells in a total volume of 0.1ml of growth medium (RPMI1640 plus 10% fetal bovine serum) are added to 96-well microtiter plates containing any variety of chemical compounds. Cells are grown for 14-17 days at 37°C in 5%CO_2. Cells are then lysed in the growth medium with 50uls of lysis buffer containing 0.1 M Tris buffer (pH 8.0), 0.1% Triton X-100, 45 mM 2-mercaptoethanol, 1mM $MgCl_2$, 0.1 M $NaPO_4$ and 0.6 mg/ml Chlorophenol-red- β-D-galactopyranoside (CPRG, Roche). Reactions are incubated for 1 hour, terminated by the addition of 50 µls of 0.5 M Na_2CO_3, and analyzed by spectrophotometry at 576 nm.

Experimental wells are compared to untreated or vehicle treated wells to identify those with increased β-galactosidase activity. Compounds producing MMR blocking activity are then further analyzed using different cell lines containing the pCAR-OF plasmid to measure the ability to block MMR as determined by MI in MMR proficient hosts by analyzing endogenous microsatellites for instability using assays described below.

EXAMPLE 3: Defining MMR blocking chemicals.

The identification of chemical inhibitors of MMR can be difficult in determining those that are standard mutagens from those that induce genomic instability via the blockade of MMR. This Example teaches of a method for determining blockers of MMR from more general mutagens. Once a compound has been identified in the assay described above, one can determine if the compound is a general mutagen or a speific MMR blocker by monitoring mutation rates in MMR proficient cells and a controlled subclone that is MMR defective. One feature of MMR deficiency is the increased resistance to toxicity of DNA alkylating agents that allows for enhanced rates of mutations upon mutagen exposure (Liu, L., et.al. *Cancer Res* (1996) 56:5375-5379). This unique feature allows for the use of a MMR proficient cell and a controlled line to measure for enhanced activity of a chemical compound to induce mutations in MMR proficient vs MMR deficient lines. If the compound is a true inhibitor of MMR then genetic mutations should occur in MMR proficient cells while no "enhanced " mutation rate will be found in already MMR defective cells. Using these criteria chemicals such as ICR191, which induces frameshift mutations in mammalian cells would not be considered a MMR

reporter construct used is called pCAR-OF, which contains a hygromycin resistance (HYG) gene plus a β-galactosidase gene with a 29 bp out-of-frame poly-CA tract inserted at the 5' end of its coding region. The pCAR-OF reporter cannot generate β-galactosidase activity unless a frame-restoring mutation (*i.e.*, insertion or deletion) arises following transfection. This line has been shown to be sensitive to inactivated MMR where using a dominant negative MMR gene allele has found this condition to result in the production of β-galactosidase (unpublished data). An example of these data using the dominant negative PMS134 allele is shown in Table 1. Briefly, H36 cells were each transfected with an expression vector containing the PMS134 allele (referred to as HB134) or empty vector and the pCAR-OF vector in duplicate reactions using the protocol below. The PMS134 gene is cloned into the pEF expression vector, which contains the elongation factor promoter upstream of the cloning site followed by a mammalian polyadenylation signal. This vector also contains the NEOr gene that allows for selection of cells in G418 to identify those retaining this plasmid. Briefly, cells were transfected with 1 μg of the PMS134 or empty vector using polyliposomes following the manufacturer's protocol (Life Technologies). Cells were then selected in 0.5 mg/ml of G418 for 10 days and G418 resistant cells were pooled together to analyze for gene expression. PMS134 positive cells, which were determined by RT-PCR and western blot (not shown) were expanded and transfected with the pCAR-OF reporter gene that contains a hygromycin (HYG) resistance gene as reporter using the protocol described above. Cells were selected in 0.5 mg/ml G418 and 0.5mg/ml HYG to select for cells retaining both the MMR effector and the pCAR-OF reporter plasmids. All cultures transfected with the pCAR vector resulted in a similar number of HYG/G418 resistant cells. Cultures were then expanded and tested for β-galactosidase activity *in situ* as well as by biochemical analysis of cell extracts. For *in situ* analysis, 100,000 cells were harvested and fixed in 1% gluteraldehyde, washed in phosphate buffered saline solution and incubated in 1 ml of X-gal substrate solution [0.15 M NaCl, 1 mM $MgCl_2$, 3.3 mM $K_4Fe(CN)_6$, 3.3 mM $K_3Fe(CN)_6$, 0.2% X-Gal] in 24 well plates for 2 hours at 37°C. Reactions were stopped in 500 mM sodium bicarbonate solution and transferred to microscope slides for analysis. Three fields of 200 cells each were counted for blue (β-galactosidase positive cells) or white (β-galactosidase negative cells) to assess for MMR inactivation. Table 1 shows the results from these studies.

blocking compound because of its ability to produce enhanced mutation rates in already MMR defective cell lines (Chen, W.D., et.al. *J Natl Cancer Inst.* (2000) 92:480-485). These screening lines include the but are not limited those in which a dominant negative MMR gene has been introduced such as that described in EXAMPLE 1 or those in which naturally MMR deficient cells such as HCT116 has been cured by introduction of a complementing MMR gene as described (Chen, W.D., et.al. *J Natl Cancer Inst.* (2000) 92:480-485).

EXAMPLE 4: Identification of chemical inhibitors of MMR *in vivo*.

MMR is a conserved post replicative DNA repair mechanism that repairs point mutations and insertion/deletions in repetitive sequences after cell division. The MMR requires an ATPase activity for initiation complex recognition and DNA translocation. *In vitro* assays have shown that the use of nonhydrolyzable forms of ATP such as AMP-PNP and ATP[gamma]S block the MMR activity (Galio, L. *et al.* (1999) *Nucl. Acids Res.* 27:2325-2331; Allen, D.J. *et al.* (1997) *EMBO J.* 16:4467-4476; Bjornson K.P. *et al.* (2000) *Biochem.* 39:3176-3183).

The use of chemicals to inhibit endogenous MMR *in vivo* has not been distinguished in the public domain. In an attempt to identify chemicals that can inhibit MMR *in vivo*, we used our H36pCAR-OF screening assay to screen for chemicals that are able to cause microsatellite instability and restoration of β-galactosidase activity from the pCAR-OF vector, an effect that can only be caused due to MMR deficiency. In our screening assays we used a variety of classes of compounds ranging from steroids such as pontasterone to potent alkylating agents such as EMS, to kinase and other enzyme inhibitors. Screens identified one class of chemicals that were capable of generating β-galactosidase positive cells. These molecules were derived from the anthracene class. An example of one such anthracene derivative for the purposes of this application is a molecule called 9,10-dimethylanthracene, referred to from here on as DMA. Fig. 3 shows the effect of DMA in shifting the pCAR-OF reporter plasmid. In contrast, general DNA alkylating agents such as EMS or MNNG did not result in MI and/or the shifting of the polynulceotide tract in the pCAR-OF reporter.

The most likely explanation for the differences in β-galactosidase activity was that the DMA compound disturbed MMR activity, resulting in a higher frequency of mutation

within the pCAR-OF reporter and re-establishing the ORF. To directly test the hypothesis that MMR was altered, we employ a biochemical assay for MMR with the individual clones as described by Nicolaides *et al.*, 1997 (Nicolaides, N.C. *et al.* (1998) *Mol. Cell. Biol.* 18:1635-1641). Nuclear extracts are prepared from the clones and incubated with heteroduplex substrates containing either a /CA\ insertion-deletion or a G/T mismatch under conditions described previously. The /CA\ and G/T heteroduplexes are used to test repair from the 3' and 5' directions, respectively as described (Nicolaides, N.C. *et al.* (1998) *Mol. Cell. Biol.* 18:1635-1641).

Biochemical assays for mismatch repair.

Enzymatic Repair Assays:

MMR activity in nuclear extracts is performed as described, using 24 fmol of substrate (Nicolaides, N.C. *et al.* (1998) *Mol. Cell. Biol.* 18:1635-1641). Complementation assays are done by adding ~ 100 ng of purified MutLa or MutSa components to 100 µg of nuclear extract, adjusting the final KCl concentration to 100 mM (Nicolaides, N.C. *et al.* (1998) *Mol. Cell. Biol.* 18:1635-1641). The substrates used in these experiments contain a strand break 181 nucleotides 5' or 125 nucleotides 3' to the mismatch.

Biochemical Activity Assays:

To demonstrate the direct effect to small molecules on MMR proteins, molecular assays such as mismatch binding and MMR complex formation are performed in the presence or absence of drug. Briefly, MMR gene cDNAs are PCR amplified using primers encompassing the entire coding regions of the known MMR proteins MSH2 (SEQ ID NO:20), GTBP (SEQ ID NO:26), MLH1 (SEQ ID NO:22), human PMS2 (SEQ ID NO:16), mouse PMS2 (SEQ ID NO:14), PMS1 (SEQ ID NO:18), and MHS3 (SEQ ID NO:28) from any species with a sense primer containing a T7 promoter and a Kozak translation signal as previously described (Nicolaides, N.C. *et al.* (1998) *Mol. Cell. Biol.* 18:1635-1641). The coding regions of known MMR proteins include the sequences shown in Table 3 for mouse *PMS2* (SEQ ID NO:15), human *PMS2* (SEQ ID NO:17), human *PMS1* (SEQ ID NO:19), human *MSH2* (SEQ ID NO:21), human *MLH1* (SEQ ID NO:23), and human *MSH3* (SEQ ID NO:29). Products are transcribed and translated using the

TNT system (Promega). An example of PCR primers and *in vitro* transcription-translation reactions are listed below.

***In vitro* transcription-translation:**

Linear DNA fragments containing *hPMS2* (SEQ ID NO:17) and *hMLH1* (SEQ ID NO:23) cDNA sequences were prepared by PCR, incorporating sequences for *in vitro* transcription and translation in the sense primer. A full-length *hMLH1* fragment was prepared using the sense primer 5'-ggatcctaatacgactcactatagggagaccaccatgtcgttcgtggcaggg-3' (SEQ ID NO:1)(codons 1-6) and the antisense primer 5'-taagtcttaagtgctaccaac-3' (SEQ ID NO:2)(located in the 3' untranslated region, nt 2411-2433), using a wild-type *hMLH1* cDNA clone as template. A full-length *hPMS2* fragment was prepared with the sense primer 5'-ggatcctaatacgactcactatagggagaccaccatggaacaattgcctgcgg-3' (SEQ ID NO:3)(codons 1-6) and the antisense primer 5'-aggttagtgaagactctgtc-3' (SEQ ID NO:4)(located in 3' untranslated region, nt 2670-2690) using a cloned *hPMS2* cDNA as template. These fragments were used to produce proteins via the coupled transcription-translation system (Promega). The reactions were supplemented with ^{35}S-labelled methionine or unlabelled methionine. Lower molecular weight bands are presumed to be degradation products and/or polypeptides translated from alternative internal methionines.

To study the effects of MMR inhibitors, assays are used to measure the formation of MLH1 and PMS2 with or without compound using polypeptides produced in the TNT System (Promega) followed by immunoprecipitation (IP). To facilitate the IP, tags may be placed at the C-terminus of the PMS2 protein to use for antibody binding or antibodies directed to the MMR protein itself can be used for IP.

Immunoprecipitations:

Immunoprecipitations are performed on *in vitro* translated proteins by mixing the translation reactions with 1 µg of the MLH1 specific monoclonal antibody (mAB) MLH14 (Oncogene Science, Inc.), a polyclonal antibody generated to codons 2-20 of hPMS2 described above, or a polyclonal antibody generated to codons 843-862 of hPMS2 (Santa Cruz Biotechnology, Inc.) in 400 µl of EBC buffer (50 mM Tris, pH 7.5, 0.1 M NaCl, 0.5% NP40). After incubation for 1 hr at 4°C, protein A sepharose (Sigma) is added to a final concentration of 10% and reactions are incubated at 4°C for 1 hour. Proteins bound

to protein A are washed five times in EBC and separated by electrophoresis on 4-20% Tris-glycine gels, which are then dried and autoradiographed.

Compounds that block heterodimerization of mutS or mutL proteins can now be identified using this assay.

EXAMPLE 5: Use of chemical MMR inhibitors yields microsatellite instability in human cells

In order to demonstrate the global ability of a chemical inhibitor of MMR in host cells and organisms, we treated human HEK293 cells (referred to as 293 cells) with DMA and measured for microsatellite instability of endogenous loci using the BAT26 diagnostic marker (Hoang J.M. *et al.* (1997) *Cancer Res.* 57:300-303). Briefly, 10^5 cells were grown in control medium or 250 μM DMA, a concentration that is found to be non-toxic, for 14 to 17 days. Cells are then harvested and genomic DNA isolated using the salting out method (Nicolaides, N.C. *et al.* (1991) *Mol. Cell. Biol.* 11:6166-6176.).

Various amounts of test DNAs from HCT116 (a human colon epithelial cell line) and 293 were first used to determine the sensitivity of our microsatellite test. The BAT26 alleles are known to be heterogeneous between these two cell lines and the products migrate at different molecular weights (Nicolaides personal observation). DNAs were diluted by limiting dilution to determine the level of sensitivity of the assay. DNAs were PCR amplified using the BAT26F: 5'-tgactacttttgacttcagcc-3' (SEQ ID NO:43) and the BAT26R: 5'-aaccattcaacatttttaaccc-3' (SEQ ID NO:44) primers in buffers as described (Nicolaides, N.C. *et al.* (1995) *Genomics* 30:195-206). Briefly 1 pg to 100 ngs of DNA were amplified using the following conditions: 94°C for 30 sec, 58°C for 30 sec, 72°C for 30 sec for 30 cycles. PCR reactions were electrophoresed on 12% polyacrylamide TBE gels (Novex) or 4% agarose gels and stained with ethidium bromide. These studies found that 0.1 ng of genomic DNA was the limit of detection using our conditions.

To measure for microsatellite stability in 293 cells grown with or without DMA, 0.1 ngs of DNA from DMA-treated or control 293 cells were amplified using the reaction conditions above. Forty individual reactions were carried out for each sample to measure for minor alleles. Fig. 4A shows a typical result from these studies whereby BAT26 alleles were amplified from DMA-treated and untreated cells and analyzed on 12% PAGE gels (Novex). Alleles from DMA-treated cells showed the presence of an altered allele

(asterisk) that migrated differently from the wild type allele. No altered alleles were found in the MMR-proficient control cells as expected since MI only occurs in MMR defective cell hosts. To confirm these data, PCRs were repeated using isolated BAT26 products. Primers and conditions were the same as described above except that reactions were amplified for 20 cycles. PCR products were gel-purified and cloned into T-tailed vectors (InVitrogen) as suggested by the manufacturer. Recombinant clones from DMA-treated and control cells were screened by PCR again using the BAT26 primers. Fifty bacterial colonies were analyzed for BAT26 structure by directly adding an aliquot of live bacteria to the PCR mix. PCR reactions were carried out as described above, and products were electrophoresed on 4% agarose gels and stained with ethidium bromide. As shown in Figure 4B, microsatellites from DMA-treated cells had alterations (asterisks) that made the marker length larger or smaller than the wild type allele found in control cells.

To confirm that these differences in molecular weight were due to shifts within the polynucleotide repeat, a hallmark of defective MMR, five clones from each sample were sequenced using an ABI automated sequencer with an M13-R primer located in the T-tail vector backbone. Sequence analysis revealed that the control cell clone used in our studies was homozygous for the BAT26 allele with a 26nt polyA repeat. Cells treated with DMA found multiple alleles ranging from the wild-type with 26 polyA repeat to shorter alleles (24 polyA repeat) and larger alleles (28 polyA repeat) (Fig. 5).

These data corroborate the H36pCAR data in Example 1 and Fig. 3 and demonstrates the ability to block MMR with a chemical in a range of hosts.

Example 6: Chemical inhibitors of MMR generate DNA hypermutability in Plants and new phenotypes.

To determine if chemical inhibitors of MMR work across a diverse array of organisms, we explored the activity of DMA on *Arabidopsis thaliana* (AT), a member of the mustard plant family, as a plant model system to study the effects of DMA on generating MMR deficiency, genome alterations, and new output traits.

Briefly, AT seeds were sterilized with straight commercial bleach and 100 seeds were plated in 100mm Murashige and Skoog (MS) phytagar (Life Technology) plates with increasing amounts of DMA (ranging from 100μm to 50mM). A similar amount of seeds were plated on MS phytagar only or in MS phytagar with increasing amounts of EMS

(100μM to 50mM), a mutagen commonly used to mutate AT seeds (McCallum, C.M.*et al.* (2000) *Nat. Biotechnol.*18:455-457). Plates were grown in a temperature-controlled, fluorescent-lighted humidifier (Percival Growth Chamber) for 10 days. After 10 days, seeds were counted to determine toxicity levels for each compound. Table 2 shows the number of healthy cells/treatment as determined by root formation and shoot formation. Plantlets that were identical to untreated seeds were scored healthy. Seeds with stunted root or shoot formation were scored intermediate (inter). Non-germinated seeds were scored dead.

Table 2: Toxicity curve of DMA and EMS on *Arabidopsis* (per 100 cells)

	0	0.1	0.5	1.0	2.5	5.0	10	12.5	25	50
DMA										
Healthy	100	94	99	99	80	85	65	0	0	0
Inter	0	0	0	0	20	15	32	85	100	0
Dead	0	0	0	0	0	0	0	0	0	100
EMS										
Healthy	99	100	45	25	0	0	0	0	0	0
Inter	0	0	54	75	0	0	0	0	0	0
Dead	0	0	0	0	100	100	100	100	100	87

The data in Table 2 show that DMA toxicity occurs at 10mM of continuous culture, while toxicity occurs at 250 μM for EMS. Next, 50 seeds were plated in two 150mm dishes containing 2mM DMA, 250 μM EMS or no drug. Seeds were grown for 10 days and then 10 plants from each plate were transferred to soil. All plants appeared to be similar in color and height. Plants were grown at room temperature with daily cycles of 18 hr light and 6 hr dark. After 45 days seeds are harvested from siliques and stored in a desiccator at 4°C for 72 hours. Seeds are then sterilized and 100 seeds from each plant is sown directly into water-saturated soil and grown at room temperature with daily cycles of 18 hr light and 6 hr dark. At day 10 phenotypically distinct plants were found in 7 out of 118 DMA treated while no phenotypic difference was observed in 150 EMS-treated or 150 control plants. These 7 altered plants were light green in color and appeared to grow

slower. Fig. 6 shows a typical difference between the DMA altered plant and controls. DMA-exposed plants produced offspring that were yellow in appearance in contrast to dark green, which is always found in wild-type plants. In addition, the yellow plants were also shorter. After 30 days, most wild-type plants produced flowers and siliques, while the 7 mutants just began flowering. After 45 days, control plants were harvested while mutant plants were harvested 10 to 15 days later. No such effects were observed in 150 plantlets from EMS treated plants.

The effect of DMA on MMR was confirmed by monitoring the structure of endogenous polynucleotide repeat markers within the plant genome. DNA was extracted using the DNAzol method following the manufacturer's protocol (Life Technology). Briefly, two leaves were harvested from DMA, EMS or untreated plants and DNA was extracted. DNAs were quantified by optical density using a BioRad Spectrophotometer. In *Arabidopsis*, a series of poly-A $(A)_n$, $(CA)_n$ and $(GA)_n$ markers were found as a result of EMBL and GenBank database searches of DNA sequence data generated as a result of the *Arabidopsis* genome-sequencing project. Two markers that are naturally occurring, ATHACS and Nga128 are used to monitor microsatellite stability using primers described (Bell, C.J. and J.R. Ecker (1994) *Genomics* 19:137-144). ATHACS has a stretch of thirty-six adenine repeats $(A)_{36}$ whereas Nga128 is characterized by a di-nucleotide AG repeat that is repeated nineteen times $(AG)_{19}$ while the Nga280 marker contains a polyAG repeat marker with 15 dinucleotides. DMA-mediated alterations of these markers are measured by a PCR assay. Briefly, the genomic DNA is amplified with specific primers in PCR reaction buffers described above using 1-10ng plant genomic DNA. Primers for each marker are listed below:

nga280:
nga280-F: 5'-CTGATCTCACGGACAATAGTGC-3' (SEQ ID NO:5)
nga280-R: 5'-GGCTCCATAAAAAGTGCACC-3' (SEQ ID NO:6)

nga128:
nga128-F: 5'-GGTCTGTTGATGTCGTAAGTCG-3' (SEQ ID NO:7)
nga128-R: 5'-ATCTTGAAACCTTTAGGGAGGG-3' (SEQ ID NO:8)

ATHACS:
ATHACS-F: 5'-AGAAGTTTAGACAGGTAC-3' (SEQ ID NO:9)
ATHACS-R: 5'-AAATGTGCAATTGCCTTC-3' (SEQ ID NO:10)

Cycling conditions are 94°C for 15 seconds, 55°C for 15 seconds and 72°C for 30 seconds, conditions that have been demonstrated to efficiently amplify these two markers (personal observation, Morphotek). PCR products are analyzed on 3.5% metaphor agarose gel in Tris-Acetate-EDTA buffer following staining with ethidium bromide.

Another method used to demonstrate that biochemical activity of a plant host's MMR is through the use of reporter genes disrupted by a polynucleotide repeat, similar to that described in Example 1 and Fig. 1. Due to the high endogenous β-galactosidase background, we engineered a plant compatible MMR-sensitive reporter gene consisting of the β-glucoronidase (GUS) gene with a mononucleotide repeat that was inserted just downstream of the initiation codon. Two repoiter constructs were generated. pGUS-OF, contained a 20 base adenine repeat inserted just downstream of the initiating methionine that resulted in a frameshift, therefore producing a nonfunctional enzyme. The second, pGUS-IF, contained a 19 base adenine repeat that retained an open reading frame and served as a control for β-glucoronidase activity. Both constructs were generated by PCR using the pBI-121 vector (Life Technologies) as template. The antisense primer was directed to the 3' end of the Nopaline Synthase (NOS) polytermination sequence contained within the pBI-121 plasmid and contained a unique *Eco*RI restriction site to facilitate cloning of the vector into the pBI-121 binary vector backbone. The sense primers contained a unique *Bam*HI restriction site to facilitate cloning of the chimeric GUS reporter gene into the pBI-121 binary vector backbone. The primers used to generate each reporter are:

1. sense primer for pGUS-IF (uidA-ATG-polyA-IF):
5'- CCC GGA TCC ATG TTA AAA AAA AAA AAA AAA AAA CGT CCT GTA GAA ACC-3' (SEQ ID NO:11)

2. sense primer for pGUS-OF (uidA-ATG-polyA-OF):
5'- CCC GGA TCC ATG TTA AAA AAA AAA AAA AAA AAA ACG TCC TGT AGA AAC C-3' (SEQ ID NO:12)

3. antisense primer (Nos-term):
5'- CCC GAA TTC CCC GAT CTA GTA ACA TAG ATG-3' (SEQ ID NO:13)

PCR amplifications were carried out using reaction buffers described above. Reactions were performed using 1 ng of pBI-121 vector as template (Life Technologies) and the appropriate corresponding primers. Amplifications were carried at 94°C for 30

seconds, 54°C for 60 seconds and 72°C for 60 seconds for 25 cycles. PCR products of the expected molecular weight was gel purified, cloned into T-tailed vectors (InVitrogen), and sequenced to ensure authentic sequence using the following primers: CaMV-FORW. [= 5'-gat atc tcc act gac gta ag-3'] (SEQ ID NO:30) for sequencing from the CaMV promoter into the 5' end of GUS cDNAs; NOSpA-42F [= 5'-tgt tgc cgg tct tgc gat g-3'] (SEQ ID NO:31) for sequencing of the NOS terminator; NOSpA-Cend-R [= 5'-ccc gat cta gta aca tag atg-3'] (SEQ ID NO:32) for sequencing from the NOS terminator into the 3' end of the GUS cDNAs; GUS-63F [= 5'-cag tct gga tcg cga aaa ctg-3'] (SEQ ID NO:33), GUS-441F [= 5'-ggt gat tac cga cga aaa cg-3'] (SEQ ID NO:34), GUS-825F [= 5'-agt gaa ggg cga aca gtt cc-3'] (SEQ ID NO:35), GUS-1224F [= 5'-gag tat tgc caa cga acc-3'] (SEQ ID NO:36), GUS-1596F [= 5'-gta tca ccg cgt ctt tga tc-3'] (SEQ ID NO:37), GUS-265R [= 5'-cga aac gca gca cga tac g-3'] (SEQ ID NO:38), GUS-646R [= 5'-gtt caa cgc tga cat cac c-3'] (SEQ ID NO:39), GUS-1033R [= 5'-cat gtt cat ctg ccc agt cg-3'] (SEQ ID NO:40), GUS-1425R [= 5'-gct ttg gac ata cca tcc-3'] (SEQ ID NO:41), and GUS-1783R [= 5'-cac cga agt tca tgc cag-3'] (SEQ ID NO:42) for the sequence of the full length GUS cDNAs. No mutation were found in either the OF or IF version of the GUS cDNA, and the expected frames for both cDNAs were also confirmed. pCR-IF-GUS and pCR-OF-GUS plasmids were subsequently digested with the BamH I and EcoR I restriction endonucleases, to generate DNA fragments containing the GUS cDNA along with the NOS terminator. These fragments were ligated into the BamH I and the EcoR I sites of the pBI-121 plasmid, which was prepared for cloning by cutting it with the same enzymes to release the wild type GUS cDNA. The resulting constructs (pBI-IF-GUS and pBI-OF-GUS) were subsequently digested with Hind III and EcoR I to release the DNA fragments encompassing the CaMV promoter, the IF or OF GUS cDNA, and the NOS terminator. Finally, these fragments were ligated into the correspondent restriction sites present in the pGPTV-HPT binary vector (ATCC) to obtain the pCMV-IF-GUS-HPT and pCMV-OF-GUS-HPT binary vectors.

The resulting vectors, CMV-OF-GUS-HPT and CMV-IF-GUS-HPT now contain the CaMV35S promoter from the Cauliflower Mosaic 35 S Virus driving the GUS gene followed by a NOS terminator and polyadenylation signal (Fig. 7). In addition, this vector also contains a hygromycin resistance gene as a selectable marker that is used to select for plants containing this reporter.

Generation of GUS reporter-expressing *Arabidopsis thaliana* transgenic plants.

Agrobacterium tumefaciens bacteria are used to shuttle binary expression vectors into plants. To generate β-glucoronidase-expressing *Arabidopsis thaliana* (*A. thaliana*) plants, *Agrobacterium tumefaciens* cells (strain GV3101) were electroporated with the CMV-OF-GUS-HPT or the CMV-IF-GUS-HPT binary vector using methods known by those skilled in the art. Briefly, one-month old *A. thaliana* (ecotype Columbia) plants were infected by immersion in a solution containing 5% sucrose, 0.05% silwet and binary vector-transformed *Agrobacteria* cells for 10 seconds. These plants were then grown at 25°C under a 16 hour day and 8 hour dark photoperiod. After 4 weeks, seeds (referred to as T1) were harvested and dried for 5 days. Thirty thousands seeds from ten CMV-OF-GUS-HPT or CMV-IF-GUS-HPT-transformed plants were sown in solid Murashige and Skoog (MS) media plates in the presence of 20 μg/ml of hygromycin (HYG). Three hundred plants were found to be HYG resistant and represented GUS expressing plants. These plants along with 300 control plants were grown in MS media for two weeks and then transferred to soil. Plants were grown for an additional four weeks under standard conditions at which time T2 seeds were harvested.

To confirm the integration and stability of the GUS vector in the plant genome, gene segregation and PCR analyses were conducted. Commonly, three out of four T1 plants transformed by *Agrobacteria* technology are expected to carry the vector inserted within a single locus and are therefore considered heterozygous for the integrated gene. Approximately 75% of the seeds (T2) generated from most of the T1 plants were found HYG–resistant and this in accordance with the expected 1:2:1 ratio of null (no GUS containing plants), heterozygous, and homozygous plants, respectively, in self-pollinating conditions. To confirm that these plants contained the GUS expression vector, genomic DNA was isolated from leaves of T1 plants using the DNAzol-mediated technique as described above. One ng of genomic DNA was analyzed by polymerase chain reaction (PCR) to confirm the presence of the GUS vector. PCR was carried out for 25 cycles with the following parameters: 95°C for 30 seconds; 54°C for 1 minute; and 72°C for 2 minutes using primers listed above. Positive reactions were observed in DNA from CMV-OF-GUS-HPT and CMV-IF-GUS-HPT-transformed plants and not from control (uninfected) plants.

In order to assess the expression of the GUS in T1 plants, leaf tissue was collected from T1 plants, homogenized in liquid nitrogen using glass pestles, and suspended in RLT lysing buffer (Qiagen, RNeasy plant RNA extraction kit). Five micrograms of total RNA was purified according to the manufacturer's suggested protocol and then loaded onto a 1.2% agarose gel (1x MOPS buffer, 3% formaldehyde), size-fractionated by electrophoresis, and transferred onto N-Hybond+ membrane (Amersham). Each membrane was incubated at 55°C in 10 ml of hybridization solution (North2South labeling kit, Pierce) containing 100 ng of GUS, tubulin, or HYG probes, which were generated by PCR amplification, according to the manufacturer's directions. Membranes were washed three times in 2x SSC, 0.1% SDS at 55°C, and three times in 2x SSC at ambient temperature. Detection was carried out using enhanced chemiluminescence (ECL). GUS message was detected in three out of ten analyzed transgenic lines, while no signal was found in the control plants. Collectively these studies demonstrated the generation of GUS expressing transgenic *A. thaliana* plants.

To determine the status of MMR activity in host plants, one can measure for the production of functional β-glucoronidase by staining plant leaves or roots *in situ* for β-glu activity. Briefly, plant tissue is washed twice with water and fixed in 4 mls of 0.02% glutaraldehyde for 15 minutes. Next, tissue is rinsed with water and incubated in X-glu solution [0.1M $NaPO_4$, 2.5 mM $K_3Fe(CN)_6$, 2.5mM $K_4Fe(CN)_6$, 1.5 mM $MgCl_2$, and 1 mg/ml X-GLU (5 bromo-4-chloro-3-indoyl- β-D-glucuronide sodium salt) (Gold Biotechnology)] for 6 hours at 37°C. Tissues are then washed twice in phosphate buffered saline (PBS) solution, once in 70% ethanol and incubated for 4 hours in methanol:acetone (3:1) for 8 hours to remove chlorophyll. Tissues are then washed twice in PBS and stored in PBS with 50% glycerol. Plant tissue with functional GUS activity will stain blue.

The presence of GUS activity in CMV-IF-GUS-HPT plants indicates that the in-frame N-terminus insertion of the poly A repeat does not disrupt the GUS protein function. The CMV-OF-GUS-HPT plants treated with DMA, EMS or untreated are tested to determine if these plants produce GUS activity. The presence of GUS activity in DMA treated plants indicates that the polyA repeat was altered, therefore, resulting in a frame-restoring mutation. Agents such as EMS, which are known to damage DNA by alkylation cannot affect the stability of a polynucleotide repeat. This data indicates that plants are defective for MMR, the only process known to be responsible for MI.

These data demonstrate the utility and power of using a chemical inhibitor of MMR to generate a high degree of genetic alteration that is not capable by means of standard DNA damaging drugs. Moreover, this application teaches of the use of reporter genes such as GUS-OF in plants to monitor for the MMR activity of a plant host.

EXAMPLE 7: Use of chemical MMR inhibitors yields microsatellite instability in microbes.

To demonstrate the ability of chemical inhibitors to block MMR in a wide range of hosts, we employed the use of *Pichia* yeast containing a pGUS-OF reporter system similar to that described in Example 5. Briefly, the GUS-OF and GUS-IF gene, which contains a polyA repeat at the N-terminus of the protein was subcloned from the pCR-IF-GUS and pCR-OF-GUS plasmids into the EcoRI site of the pGP vector, which is a consitutively expressed yeast vector containing a zeocin resistance gene as selectable marker. pGP-GUS-IF and pGP-GUS-OF vectors were electroporated into competent *Pichia* cells using standard methods known by those skilled in the art. Cells were plated on YPD agar (10g/L yeast extract; 20 g/L peptone; 2% glucose; 1.5% bactoagar) plates containing 100 μg/ml zeocin. Recombinant yeast are then analyzed for GUS expression/function by replica plating on YPD agar plates containing 100 μg/ml zeocin plus 1 mg/ml X-glu (5-bromo-4-chloro-3-indoyl-beta-D-glucuronide sodium salt) and grown at 30°C for 16 hours. On hundred percent of yeast expressing GUS-IF were found to turn blue in the presence of the X-glu substrate while none of the control yeast turned blue. None of the yeast containing the GUS-OF turned blue in the presence of the X-glu substrate under normal growth conditions.

To demonstrate the ability of chemicals to block MMR in yeast, GUS-OF and control cells were incubated with 300 μM DMA, EMS, or no chemical for 48 hours. After incubation, yeast were plated on YPD-ZEO-X-GLU plates and grown at 30°C for 16 hours. After incubation, a subset of yeast expressing GUS-OF contain blue subclones, while none are seen in EMS or control cells. These data demonstrate the ability of chemicals to block MMR of microbes *in vivo* to produce subclones with new output traits.

EXAMPLE 8: Classes of other chemicals capable of blocking MMR in vivo

The discovery of anthracene compounds presents a new method for blocking MMR activity of host organisms *in vivo*. While 9,10-dimethylanthracene (DMA) was found to block MMR in cell hosts, other analogs with a similar chemical composition from this class are also claimed in this invention. These include anthracene and related analogs such as 9,10-diphenylanthracene and 9,10-di-M-tolylanthracene. Myers *et al.* ((1988) *Biochem. Biophys. Res. Commun.* 151:1441-1445) disclosed that at high concentrations, DMA acts as a potent weak mutagen, while metabolized forms of DMA are the "active" ingredients in promoting mutation. This finding suggests that metabolites of anthracene-based compounds may also act as active inhibitors of MMR *in vivo*. For instance, metabolism of anthracene and 9,10-dimethylanthracene by *Micrococcus sp.*, *Pseudomonas sp.* and *Bacillus macerans* microbes have found a number of anthracene and 9,10-dimethylanthracene metabolites are formed. These include anthracene and 9,10-dimethylanthracene cis-dihydrodiols, hydroxy-methyl-derivatives and various phenolic compounds. Bacteria metabolize hydrocarbons using the dioxygenase enzyme system, which differs from the mammalian cytochrome P-450 monoxygenase. These findings suggest the use of bacteria for biotransforming anthracene and DMA for additional MMR blocking compounds (Traczewska, T.M. *et al.* (1991) *Acta. Microbiol. Pol.* 40:235-241). Metabolism studies of DMA by rat-liver microsomal preparations has found that this molecule is converted to 9-Hydroxymethyl-10-methylanthracene (9-OHMeMA) and 9,10-dihydroxymethyl-anthracene (9,10-DiOHMeA) (Lamparczyk, H.S. *et al.* (1984) *Carcinogenesis* 5:1405-1410). In addition, the trans-1,2-dihydro-1,2-dihydroxy derivative of DMA (DMA 1,2-diol) was found to be a major metabolite as determined by chromatographic, ultraviolet (UV), nuclear magnetic resonance (NMR), and mass spectral properties. DMA 1,2-diol was also created through the oxidation of DMA in an ascorbic acid-ferrous sulfate-EDTA system. Other dihydrodiols that are formed from DMA by metabolism are the trans-1,2- and 3,4-dihydrodiols of 9-OHMeMA (9-OHMeMA 1,2-diol and 9-OHMeMA 3,4-diol) while the further metabolism of DMA 1,2-diol can yield both of these dihydrodiols. Finally, when 9-OHMeMA is further metabolized, two main metabolites are formed; one was identified as 9,10-DiOHMeA and the other appeared to be 9-OHMeMA 3,4-diol.

The metabolism of 9-methylanthracene (9-MA), 9-hydroxymethylanthracene (9-OHMA), and 9,10-dimethylanthracene (9,10-DMA) by fungus also has been reported (Cerniglia, C.E. *et al.* (1990) *Appl. Environ. Microbiol.* 56:661-668). These compounds are also useful for generating DMA derivatives capable of blocking MMR. Compounds 9-MA and 9,10-DMA are metabolized by two pathways, one involving initial hydroxylation of the methyl group(s) and the other involving epoxidation of the 1,2- and 3,4- aromatic double bond positions, followed by enzymatic hydration to form hydroxymethyl trans-dihydrodiols. For 9-MA metabolism, the major metabolites identified are trans-1,2-dihydro-1,2-dihydroxy and trans-3,4-dihydro-3,4-dihydroxy derivatives of 9-MA and 9-OHMA, whereby 9-OHMA can be further metabolized to trans-1,2- and 3,4-dihydrodiol derivatives. Circular dichroism spectral analysis revealed that the major enantiomer for each dihydrodiol was predominantly in the S,S configuration, in contrast to the predominantly R,R configuration of the trans-dihydrodiol formed by mammalian enzyme systems. These results indicate that *Caenorhabditis elegans* metabolizes methylated anthracenes in a highly stereoselective manner that is different from that reported for rat liver microsomes.

The analogs as listed above provide an example but are not limited to anthracene-derived compounds capable of eliciting MMR blockade. Additional analogs that are of potential use for blocking MMR are shown in Fig.8.

Other classes of small molecular weight compounds that are capable of blocking MMR *in vivo*.

MMR is a multi-step process that involves the formation of protein complexes that detect mismatched bases or altered repetitive sequences and interface these mutations with enzymes that degrade the mutant base and repair the DNA with correct nucleotides. First, mismatched DNA is recognized by the mutS heterodimeric complex consisting of MSH2 and GTBP proteins. The DNA bound mutS complex is then recognized by the mutL heterdimeric complex that consists of PMS2 and MLH1 proteins. The mutL complex is thought to interface exonucleases with the mismatched DNA site, thus initiating this specialized DNA repair process. After the mismatched bases are removed, the DNA is repaired with a polymerase.

There are several steps in the normal process that can be targeted by small molecular weight compounds to block MMR. This application teaches of these steps and the types of compounds that may be used to block this process.

ATPase inhibitors:

The finding that nonhydrolyzable forms of ATP are able to suppress MMR *in vitro* also suggest that the use for this type of compound can lead to blockade of MMR *in vivo* and mutation a host organism's genome (Galio, L. *et al.* (1999) *Nucl. Acids Res.* 27:2325-2331; Allen, D.J. *et al.* (1997) *EMBO J.* 16:4467-4476; Bjornson, K.P. *et al.* (2000) *Biochem.* 39:3176-3183). One can use a variety of screening methods described within this application to identify ATP analogs that block the ATP-dependent steps of mismatch repair *in vivo*.

Nuclease inhibitors:

The removal of mismatched bases is a required step for effective MMR (Harfe, B.D. and S. Jinks-Robertson (2000) *Ann. Rev. Genet.* 34:359-399). This suggests that compounds capable of blocking this step can lead to blockade of MMR *in vivo* and mutation a host organism's genome. One can use a variety of screening methods described within this application to identify nuclease inhibitors analogs that block the nuclease steps of mismatch repair *in vivo*. An example of the types of nuclease inhibitors are but not limited to analogs of N-Ethylmaleimide, an endonuclease inhibitor (Huang, Y.C., et.al. (1995) *Arch. Biochem. Biophys.* 316:485), heterodimeric adenine-chain-acridine compounds, exonulcease III inhibitors (Belmont P, et.al., *Bioorg Med Chem Lett* (2000) 10:293-295), as well as antibiotic compounds such as Heliquinomycin, which have helicase inhibitory activity (Chino, M, et.al. *J. Antibiot. (Tokyo)* (1998) 51:480-486).

Polymerase inhibitors:

Short and long patch repair is a required step for effective MMR (Modrich, P. (1994) *Science* 266:1959-1960). This suggests that compounds capable of blocking MMR-associated polymerization can lead to blockade of MMR *in vivo* and mutation a host organism's genome. One can use a variety of screening methods described within this application to identify polymerase inhibitors analogs that block the polymerization steps of

mismatch repair *in vivo*. An example of DNA polymerase inhibitors that are useful in blocking MMR activity include, but are not limited to, analogs of actinomycin D (Martin, S.J., et.al. (1990) *J. Immunol.* 145:1859), Aphidicolin (Kuwakado, K. et.al. (1993) *Biochem. Pharmacol.* 46:1909) 1-(2'-Deoxy-2'-fluoro-beta-L-arabinofuranosyl)-5-methyluracil (L-FMAU) (Kukhanova M, et.al., *Biochem Pharmacol* (1998) 55:1181-1187), and 2',3'-dideoxyribonucleoside 5'-triphosphates (ddNTPs) (Ono, K., et.al., *Biomed Pharmacother* (1984) 38:382-389).

Chemical Inhibitors of Mismatch Repair Gene Expression

MMR is a multi-protein process that requires the cooperation of several proteins such as but not limited to mutS homologs, MSH2, MSH3, MSH6, GTBP; mutL homologs PMS1, PMS2, MLH1; and exonucleases and helicases such as MutH and MutY (Harfe, B.D. and S. Jinks-Robertson (2000) *Ann. Rev. Genet.* 34:359-399; Modrich, P. (1994) *Science* 266:1959-1960). Chemicals capable of blocking the expression of these genes can lead to the blockade of MMR. An example of a chemical that is capable of blocking MMR gene expression is an oligodeoxynucleotide that can specifically bind and degrade an MMR gene message and protein production as described by Chauhan DP, et.al. *(Clin Cancer Res* (2000) 6:3827-3831). One can use a variety of screening methods described within this application to identify inhibitors that block the expression and/or function of MMR genes *in vivo*.

DISCUSSION

The results described herein demonstrate the use of chemicals that can block mismatch repair of host organisms *in vivo* to produce genetic mutations. The results also demonstrate the use of reporter systems in host cells and organisms that are useful for screening chemicals capable of blocking MMR of the host organism. Moreover, the results demonstrate the use of chemical inhibitors to block MMR in mammalian cells, microbes, and plants to produce organisms with new output traits. The data presented herein provide novel approaches for producing genetically altered plants, microbes, and mammalian cells with output traits for commercial applications by inhibiting MMR with chemicals. This approach gives advantages over others that require the use of recombinant techniques to block MMR or to produce new output traits by expression of a foreign gene.

This method will be useful in producing genetically altered host organisms for agricultural, chemical manufacturing, pharmaceutical, and environmental applications.

PMS2 (mouse) (SEQ ID NO:14)

```
MEQTEGVSTE CAKAIKPIDG KSVHQICSGQ VILSLSTAVK ELIENSVDAG ATTIDLRLKD  60
YGVDLIEVSD NGCGVEEENF EGLALKHHTS KIQEFADLTQ VETFGFRGEA LSSLCALSDV 120
TISTCHGSAS VGTRLVFDHN GKITQKTPYP RPKGTTVSVQ HLFYTLPVRY KEFQRNIKKE 180
YSKMVQVLQA YCIISAGVRV SCTNQLGQGK RHAVVCTSGT SGMKENIGSV FGQKQLQSLI 240
PFVQLPPSDA VCEEYGLSTS GRHKTFSTFR ASFHSARTAP GGVQQTGSFS SSIRGPVTQQ 300
RSLSLSMRFY HMYNRHQYPF VVLNVSVDSE CVDINVTPDK RQILLQEEKL LLAVLKTSLI 360
GMFDSDANKL NVNQQPLLDV EGNLVKLHTA ELEKPVPGKQ DNSPSLKSTA DEKRVASISR 420
LREAFSLHPT KEIKSRGPET AELTRSFPSE KRGVLSSYPS DVISYRGLRG SQDKLVSPTD 480
SPGDCMDREK IEKDSGLSST SAGSEEEFST PEVASSFSSD YNVSSLEDRP SQETINCGDL 540
DCRPPGTGQS LKPEDHGYQC KALPLARLSP TNAKRFKTEE RPSNVNISQR LPGPQSTSAA 600
EVDVAIKMNK RIVLLEFSLS SLAKRMKQLQ HLKAQNKHEL SYRKFRAKIC PGENQAAEDE 660
LRKEISKSMF AEMEILGQFN LGFIVTKLKE DLFLVDQHAA DEKYNFEMLQ QHTVLQAQRL 720
ITPQTLNLTA VNEAVLIENL EIFRKNGFDF VIDEDAPVTE RAKLISLPTS KNWTFGPQDI 780
DELIFMLSDS PGVMCRPSRV RQMFASRACR KSVMIGTALN ASEMKKLITH MGEMDHPWNC 840
PHGRPTMRHV ANLDVISQN                                              859
```

PMS2 (mouse cDNA) (SEQ ID NO:15)

```
gaattccggt gaaggtcctg aagaatttcc agattcctga gtatcattgg aggagacaga 60
taacctgtcg tcaggtaacg atggtgtata tgcaacagaa atgggtgttc ctggagacgc 120
gtcttttccc gagagcggca ccgcaactct cccgcggtga ctgtgactgg aggagtcctg 180
catccatgga gcaaaccgaa ggcgtgagta cagaatgtgc taaggccatc aagcctattg 240
atgggaagtc agtccatcaa atttgttctg ggcaggtgat actcagttta agcaccgctg 300
tgaaggagtt gatagaaaat agtgtagatg ctggtgctac tactattgat ctaaggctta 360
aagactatgg ggtggacctc attgaagttt cagacaatgg atgtggggta gaagaagaaa 420
actttgaagg tctagctctg aaacatcaca catctaagat tcaagagttt gccgacctca 480
cgcaggttga aactttcggc tttcgggggg aagctctgag ctctctgtgt gcactaagtg 540
atgtcactat atctacctgc cacgggtctg caagcgttgg gactcgactg gtgtttgacc 600
ataatgggaa aatcacccag aaaactccct acccccgacc taaaggaacc acagtcagtg 660
tgcagcactt attttataca ctacccgtgc gttacaaaga gtttcagagg aacattaaaa 720
aggagtattc caaaatggtg caggtcttac aggcgtactg tatcatctca gcaggcgtcc 780
gtgtaagctg cactaatcag ctcggacagg ggaagcggca cgctgtggtg tgcacaagcg 840
gcacgtctgg catgaaggaa aatatcgggt ctgtgtttgg ccagaagcag ttgcaaagcc 900
tcattccttt tgttcagctg ccccctagtg acgctgtgtg tgaagagtac ggcctgagca 960
cttcaggacg ccacaaaacc ttttctacgt ttcgggcttc atttcacagt gcacgcacgg 1020
cgccgggagg agtgcaacag acaggcagtt tttcttcatc aatcagaggc cctgtgaccc 1080
agcaaaggtc tctaagcttg tcaatgaggt tttatcacat gtataaccgg catcagtacc 1140
catttgtcgt ccttaacgtt tccgttgact cagaatgtgt ggatattaat gtaactccag 1200
ataaaaggca aattctacta caagaagaga agctattgct ggccgtttta aagacctcct 1260
tgataggaat gtttgacagt gatgcaaaca agcttaatgt caaccagcag ccactgctag 1320
atgttgaagg taacttagta aagctgcata ctgcagaact agaaaagcct gtgccaggaa 1380
agcaagataa ctctccttca ctgaagagca cagcagacga gaaaagggta gcatccatct 1440
ccaggctgag agaggccttt tctcttcatc ctactaaaga gatcaagtct aggggtccag 1500
agactgctga actgacacgg agttttccaa gtgagaaaag gggcgtgtta tcctcttatc 1560
cttcagacgt catctcttac agaggcctcc gtggctcgca ggacaaattg gtgagtccca 1620
cggacagccc tggtgactgt atggacagag agaaaataga aaaagactca gggctcagca 1680
gcacctcagc tggctctgag gaagagttca gcaccccaga agtggccagt agctttagca 1740
gtgactataa cgtgagctcc ctagaagaca gaccttctca ggaaaccata aactgtggtg 1800
acctggactg ccgtcctcca ggtacaggac agtccttgaa gccagaagac catggatatc 1860
aatgcaaagc tctacctcta gctcgtctgt cacccacaaa tgccaagcgc ttcaagacag 1920
aggaaagacc ctcaaatgtc aacatttctc aaagattgcc tggtcctcag agcacctcag 1980
cagctgaggt cgatgtagcc ataaaaatga ataagagaat cgtgctcctc gagttctctc 2040
tgagttctct agctaagcga atgaagcagt tacagcacct aaaggcgcag aacaaacatg 2100
aactgagtta cagaaaattt agggccaaga tttgccctgg agaaaaccaa gcagcagaag 2160
atgaactcag aaaagagatt agtaaatcga tgtttgcaga gatggagatc ttgggtcagt 2220
ttaacctggg atttatagta accaaactga aagaggacct cttcctggtg gaccagcatg 2280
ctgcggatga gaagtacaac tttgagatgc tgcagcagca cacggtgctc caggcgcaga 2340
```

ggctcatcac accccagact ctgaacttaa ctgctgtcaa tgaagctgta ctgatagaaa 2400
atctggaaat attcagaaag aatggctttg actttgtcat tgatgaggat gctccagtca 2460
ctgaaagggc taaattgatt tccttaccaa ctagtaaaaa ctggaccttt ggaccccaag 2520
atatagatga actgatcttt atgttaagtg acagccctgg ggtcatgtgc cggccctcac 2580
gagtcagaca gatgtttgct tccagagcct gtcggaagtc agtgatgatt ggaacggcgc 2640
tcaatgcgag cgagatgaag aagctcatca cccacatggg tgagatggac caccctgga 2700
actgccccca cggcaggcca accatgaggc acgttgccaa tctggatgtc atctctcaga 2760
actgacacac cccttgtagc atagagttta ttacagattg ttcggtttgc aaagagaagg 2820
ttttaagtaa tctgattatc gttgtacaaa aattagcatg ctgctttaat gtactggatc 2880
catttaaaag cagtgttaag gcaggcatga tggagtgttc ctctagctca gctacttggg 2940
tgatccggtg ggagctcatg tgagcccagg actttgagac cactccgagc cacattcatg 3000
agactcaatt caaggacaaa aaaaaaaaga tatttttgaa gccttttaaa aaaaaa 3056

PMS2 (human) (SEQ ID NO:16)

MERAESSSTE PAKAIKPIDR KSVHQICSGQ VVLSLSTAVK ELVENSLDAG ATNIDLKLKD 60
YGVDLIEVSD NGCGVEEENF EGLTLKHHTS KIQEFADLTQ VETFGFRGEA LSSLCALSDV 120
TISTCHASAK VGTRLMFDHN GKIIQKTPYP RPRGTTVSVQ QLFSTLPVRH KEFQRNIKKE 180
YAKMVQVLHA YCIISAGIRV SCTNQLGQGK RQPVVCTGGS PSIKENIGSV FGQKQLQSLI 240
PFVQLPPSDS VCEEYGLSCS DALHNLFYIS GFISQCTHGV GRSSTDRQFF FINRRPCDPA 300
KVCRLVNEVY HMYNRHQYPF VVLNISVDSE CVDINVTPDK RQILLQEEKL LLAVLKTSLI 360
GMFDSDVNKL NVSQQPLLDV EGNLIKMHAA DLEKPMVEKQ DQSPSLRTGE EKKDVSISRL 420
REAFSLRHTT ENKPHSPKTP EPRRSPLGQK RGMLSSSTSG AISDKGVLRP QKEAVSSSHG 480
PSDPTDRAEV EKDSGHGSTS VDSEGFSIPD TGSHCSSEYA ASSPGDRGSQ EHVDSQEKAP 540
ETDDSFSDVD CHSNQEDTGC KFRVLPQPTN LATPNTKRFK KEEILSSSDI CQKLVNTQDM 600
SASQVDVAVK INKKVVPLDF SMSSLAKRIK QLHHEAQQSE GEQNYRKFRA KICPGENQAA 660
EDELRKEISK TMFAEMEIIG QFNLGFIITK LNEDIFIVDQ HATDEKYNFE MLQQHTVLQG 720
QRLIAPQTLN LTAVNEAVLI ENLEIFRKNG FDFVIDENAP VTERAKLISL PTSKNWTFGP 780
QDVDELIFML SDSPGVMCRP SRVKQMFASR ACRKSVMIGT ALNTSEMKKL ITHMGEMDHP 840
WNCPHGRPTM RHIANLGVIS QN 862

PMS2 (human cDNA) (SEQ ID NO:17)

cgaggcggat cgggtgttgc atccatggag cgagctgaga gctcgagtac agaacctgct 60
aaggccatca aacctattga tcggaagtca gtccatcaga tttgctctgg gcaggtggta 120
ctgagtctaa gcactgcggt aaaggagtta gtagaaaaca gtctggatgc tggtgccact 180
aatattgatc taaagcttaa ggactatgga gtggatctta ttgaagtttc agacaatgga 240
tgtggggtag aagaagaaaa cttcgaaggc ttaactctga aacatcacac atctaagatt 300
caagagtttg ccgacctaac tcaggttgaa acttttggct ttcgggggga agctctgagc 360
tcactttgtg cactgagcga tgtcaccatt tctacctgcc acgcatcggc gaaggttgga 420
actcgactga tgtttgatca caatgggaaa attatccaga aaacccccta cccccgcccc 480
agagggacca cagtcagcgt gcagcagtta ttttccacac tacctgtgcg ccataaggaa 540
tttcaaagga atattaagaa ggagtatgcc aaaatggtcc aggtcttaca tgcatactgt 600
atcatttcag caggcatccg tgtaagttgc accaatcagc ttggacaagg aaaacgacag 660
cctgtggtat gcacaggtgg aagccccagc ataaaggaaa atatcggctc tgtgtttggg 720
cagaagcagt tgcaaagcct cattcctttt gttcagctgc cccctagtga ctccgtgtgt 780
gaagagtacg gtttgagctg ttcggatgct ctgcataatc ttttttacat ctcaggtttc 840
atttcacaat gcacgcatgg agttggaagg agttcaacag acagacagtt tttctttatc 900
aaccggcggc cttgtgaccc agcaaaggtc tgcagactcg tgaatgaggt ctaccacatg 960
tataatcgac accagtatcc atttgttgtt cttaacattt ctgttgattc agaatgcgtt 1020
gatatcaatg ttactccaga taaaaggcaa attttgctac aagaggaaaa gcttttgttg 1080
gcagttttaa agacctcttt gataggaatg tttgatagtg atgtcaacaa gctaaatgtc 1140
agtcagcagc cactgctgga tgttgaaggt aacttaataa aaatgcatgc agcggatttg 1200
gaaaagccca tggtagaaaa gcaggatcaa tccccttcat taaggactgg agaagaaaaa 1260
aaagacgtgt ccatttccag actgcgagag gccttttctc ttcgtcacac aacagagaac 1320
aagcctcaca gcccaaagac tccagaacca agaaggagcc ctctaggaca gaaaaggggt 1380
atgctgtctt ctagcacttc aggtgccatc tctgacaaag gcgtcctgag acctcagaaa 1440
gaggcagtga gttccagtca cggacccagt gaccctacgg acagagcgga ggtggagaag 1500
gactcggggc acggcagcac ttccgtggat tctgaggggt tcagcatccc agacacgggc 1560
agtcactgca gcagcgagta tgcggccagc tccccagggg acaggggctc gcaggaacat 1620
gtggactctc aggagaaagc gcctgaaact gacgactctt tttcagatgt ggactgccat 1680
tcaaaccagg aagataccgg atgtaaattt cgagttttgc ctcagccaac taatctcgca 1740
accccaaaca caaagcgttt taaaaaagaa gaaattcttt ccagttctga catttgtcaa 1800
aagttagtaa atactcagga catgtcagcc tctcaggttg atgtagctgt gaaaattaat 1860
aagaaagttg tgcccctgga cttttctatg agttctttag ctaaacgaat aaagcagtta 1920
catcatgaag cacagcaaag tgaaggggaa cagaattaca ggaagtttag ggcaaagatt 1980

tgtcctggag aaaatcaagc agccgaagat gaactaagaa aagagataag taaaacgatg 2040
tttgcagaaa tggaaatcat tggtcagttt aacctgggat ttataataac caaactgaat 2100
gaggatatct tcatagtgga ccagcatgcc acggacgaga agtataactt cgagatgctg 2160
cagcagcaca ccgtgctcca ggggcagagg ctcatagcac ctcagactct caacttaact 2220
gctgttaatg aagctgttct gatagaaaat ctggaaatat ttagaaagaa tggctttgat 2280
tttgttatcg atgaaaatgc tccagtcact gaaagggcta aactgatttc cttgccaact 2340
agtaaaaact ggaccttcgg accccaggac gtcgatgaac tgatcttcat gctgagcgac 2400
agccctgggg tcatgtgccg gccttcccga gtcaagcaga tgtttgcctc cagagcctgc 2460
cggaagtcgg tgatgattgg gactgctctt aacacaagcg agatgaagaa actgatcacc 2520
cacatggggg agatggacca cccctggaac tgtccccatg gaaggccaac catgagacac 2580
atcgccaacc tgggtgtcat ttctcagaac tgaccgtagt cactgtatgg aataattggt 2640
tttatcgcag atttttatgt tttgaaagac agagtcttca ctaacctttt ttgttttaaa 2700
atgaaacctg ctacttaaaa aaaatacaca tcacacccat ttaaaagtga tcttgagaac 2760
cttttcaaac c 2771

PMS1 (human) (SEQ ID NO:18)

MKQLPAATVR LLSSSQIITS VVSVVKELIE NSLDAGATSV DVKLENYGFD KIEVRDNGEG 60
IKAVDAPVMA MKYYTSKINS HEDLENLTTY GFRGEALGSI CCIAEVLITT RTAADNFSTQ 120
YVLDGSGHIL SQKPSHLGQG TTVTALRLFK NLPVRKQFYS TAKKCKDEIK KIQDLLMSFG 180
ILKPDLRIVF VHNKAVIWQK SRVSDHKMAL MSVLGTAVMN NMESFQYHSE ESQIYLSGFL 240
PKCDADHSFT SLSTPERSFI FINSRPVHQK DILKLIRHHY NLKCLKESTR LYPVFFLKID 300
VPTADVDVNL TPDKSQVLLQ NKESVLIALE NLMTTCYGPL PSTNSYENNK TDVSAADIVL 360
SKTAETDVLF NKVESSGKNY SNVDTSVIPF QNDMHNDESG KNTDDCLNHQ ISIGDFGYGH 420
CSSEISNIDK NTKNAFQDIS MSNVSWENSQ TEYSKTCFIS SVKHTQSENG NKDHIDESGE 480
NEEEAGLENS SEISADEWSR GNILKNSVGE NIEPVKILVP EKSLPCKVSN NNYPIPEQMN 540
LNEDSCNKKS NVIDNKSGKV TAYDLLSNRV IKKPMSASAL FVQDHRPQFL IENPKTSLED 600
ATLQIEELWK TLSEEEKLKY EEKATKDLER YNSQMKRAIE QESQMSLKDG RKKIKPTSAW 660
NLAQKHKLKT SLSNQPKLDE LLQSQIEKRR SQNIKMVQIP FSMKNLKINF KKQNKVDLEE 720
KDEPCLIHNL RFPDAWLMTS KTEVMLLNPY RVEEALLFKR LLENHKLPAE PLEKPIMLTE 780
SLFNGSHYLD VLYKMTADDQ RYSGSTYLSD PRLTANGFKI KLIPGVSITE NYLEIEGMAN 840
CLPFYGVADL KEILNAILNR NAKEVYECRP RKVISYLEGE AVRLSRQLPM YLSKEDIQDI 900
IYRMKHQFGN EIKECVHGRP FFHHLTYLPE TT 932

PMS1 (human) (SEQ ID NO:19)

ggcacgagtg gctgcttgcg gctagtggat ggtaattgcc tgcctcgcgc tagcagcaag 60
ctgctctgtt aaaagcgaaa atgaaacaat tgcctgcggc aacagttcga ctcctttcaa 120
gttctcagat catcacttcg gtggtcagtg ttgtaaaaga gcttattgaa aactccttgg 180
atgctggtgc cacaagcgta gatgttaaac tggagaacta tggatttgat aaaattgagg 240
tgcgagataa cggggagggt atcaaggctg ttgatgcacc tgtaatggca atgaagtact 300
acacctcaaa aataaatagt catgaagatc ttgaaaattt gacaacttac ggttttcgtg 360
gagaagcctt ggggtcaatt tgttgtatag ctgaggtttt aattacaaca agaacggctg 420
ctgataattt tagcacccag tatgttttag atggcagtgg ccacatactt tctcagaaac 480
cttcacatct tggtcaaggt acaactgtaa ctgctttaag attatttaag aatctacctg 540
taagaaagca gttttactca actgcaaaaa aatgtaaaga tgaaataaaa aagatccaag 600
atctcctcat gagctttggt atccttaaac ctgacttaag gattgtcttt gtacataaca 660
aggcagttat ttggcagaaa agcagagtat cagatcacaa gatggctctc atgtcagttc 720
tggggactgc tgttatgaac aatatggaat cctttcagta ccactctgaa gaatctcaga 780
tttatctcag tggatttctt ccaaagtgtg atgcagacca ctctttcact agtctttcaa 840
caccagaaag aagtttcatc ttcataaaca gtcgaccagt acatcaaaaa gatatcttaa 900
agttaatccg acatcattac aatctgaaat gcctaaagga atctactcgt ttgtatcctg 960
ttttctttct gaaaatcgat gttcctacag ctgatgttga tgtaaattta acaccagata 1020
aaagccaagt attattacaa aataaggaat ctgttttaat tgctcttgaa aatctgatga 1080
cgacttgtta tggaccatta cctagtacaa attcttatga aaataataaa acagatgttt 1140
ccgcagctga catcgttctt agtaaaacag cagaaacaga tgtgcttttt aataaagtgg 1200
aatcatctgg aaagaattat tcaaatgttg atacttcagt cattccattc caaaatgata 1260
tgcataatga tgaatctgga aaaaacactg atgattgttt aaatcaccag ataagtattg 1320
gtgactttgg ttatggtcat tgtagtagtg aaatttctaa cattgataaa aacactaaga 1380
atgcatttca ggacatttca atgagtaatg tatcatggga gaactctcag acggaatata 1440
gtaaaacttg ttttataagt tccgttaagc acacccagtc agaaaatggc aataaagacc 1500
atatagatga gagtggggaa aatgaggaag aagcaggtct tgaaaactct tcggaaattt 1560
ctgcagatga gtggagcagg ggaaatatac ttaaaaattc agtgggagag aatattgaac 1620
ctgtgaaaat tttagtgcct gaaaaaagtt taccatgtaa agtaagtaat aataattatc 1680
caatccctga acaaatgaat cttaatgaag attcatgtaa caaaaaatca aatgtaatag 1740
ataataaatc tggaaaagtt acagcttatg atttacttag caatcgagta atcaagaaac 1800

ccatgtcagc aagtgctctt tttgttcaag atcatcgtcc tcagtttctc atagaaaatc 1860
ctaagactag tttagaggat gcaacactac aaattgaaga actgtggaag acattgagtg 1920
aagaggaaaa actgaaatat gaagagaagg ctactaaaga cttggaacga tacaatagtc 1980
aaatgaagag agccattgaa caggagtcac aaatgtcact aaaagatggc agaaaaaaga 2040
taaaacccac cagcgcatgg aatttggccc agaagcacaa gttaaaaacc tcattatcta 2100
atcaaccaaa acttgatgaa ctccttcagt cccaaattga aaaaagaagg agtcaaaata 2160
ttaaaatggt acagatcccc ttttctatga aaaacttaaa aataaatttt aagaaacaaa 2220
acaaagttga cttagaagag aaggatgaac cttgcttgat ccacaatctc aggtttcctg 2280
atgcatggct aatgacatcc aaaacagagg taatgttatt aaatccatat agagtagaag 2340
aagccctgct atttaaaaga cttcttgaga atcataaact tcctgcagag ccactggaaa 2400
agccaattat gttaacagag agtctttta atggatctca ttatttagac gttttatata 2460
aaatgacagc agatgaccaa agatacagtg gatcaactta cctgtctgat cctcgtctta 2520
cagcgaatgg tttcaagata aaattgatac caggagtttc aattactgaa aattacttgg 2580
aaatagaagg aatggctaat tgtctcccat tctatggagt agcagattta aaagaaattc 2640
ttaatgctat attaaacaga aatgcaaagg aagtttatga atgtagacct cgcaaagtga 2700
taagttattt agagggagaa gcagtgcgtc tatccagaca attacccatg tacttatcaa 2760
aagaggacat ccaagacatt atctacagaa tgaagcacca gtttggaaat gaaattaaag 2820
agtgtgttca tggtcgccca ttttttcatc atttaaccta tcttccagaa actacatgat 2880
taaatatgtt taagaagatt agttaccatt gaaattggtt ctgtcataaa acagcatgag 2940
tctggtttta aattatcttt gtattatgtg tcacatggtt atttttaaa tgaggattca 3000
ctgacttgtt tttatattga aaaaagttcc acgtattgta gaaaacgtaa ataaactaat 3060
aac 3063

MSH2 (human) (SEQ ID NO:20)

MAVQPKETLQ LESAAEVGFV RFFQGMPEKP TTTVRLFDRG DFYTAHGEDA LLAAREVFKT 60
QGVIKYMGPA GAKNLQSVVL SKMNFESFVK DLLLVRQYRV EVYKNRAGNK ASKENDWYLA 120
YKASPGNLSQ FEDILFGNND MSASIGVVGV KMSAVDGQRQ VGVGYVDSIQ RKLGLCEFPD 180
NDQFSNLEAL LIQIGPKECV LPGGETAGDM GKLRQIIQRG GILITERKKA DFSTKDIYQD 240
LNRLLKGKKG EQMNSAVLPE MENQVAVSSL SAVIKFLELL SDDSNFGQFE LTTFDFSQYM 300
KLDIAAVRAL NLFQGSVEDT TGSQSLAALL NKCKTPQGQR LVNQWIKQPL MDKNRIEERL 360
NLVEAFVEDA ELRQTLQEDL LRRFPDLNRL AKKFQRQAAN LQDCYRLYQG INQLPNVIQA 420
LEKHEGKHQK LLLAVFVTPL TDLRSDFSKF QEMIETTLDM DQVENHEFLV KPSFDPNLSE 480
LREIMNDLEK KMQSTLISAA RDLGLDPGKQ IKLDSSAQFG YYFRVTCKEE KVLRNNKNFS 540
TVDIQKNGVK FTNSKLTSLN EEYTKNKTEY EEAQDAIVKE IVNISSGYVE PMQTLNDVLA 600
QLDAVVSFAH VSNGAPVPYV RPAILEKGQG RIILKASRHA CVEVQDEIAF IPNDVYFEKD 660
KQMFHIITGP NMGGKSTYIR QTGVIVLMAQ IGCFVPCESA EVSIVDCILA RVGAGDSQLK 720
GVSTFMAEML ETASILRSAT KDSLIIIDEL GRGTSTYDGF GLAWAISEYI ATKIGAFCMF 780
ATHFHELTAL ANQIPTVNNL HVTALTTEET LTMLYQVKKG VCDQSFGIHV AELANFPKHV 840
IECAKQKALE LEEFQYIGES QGYDIMEPAA KKCYLEREQG EKIIQEFLSK VKQMPFTEMS 900
EENITIKLKQ LKAEVIAKNN SFVNEIISRI KVTT 934

MSH2 (human cDNA) (SEQ ID NO:21)

ggcgggaaac agcttagtgg gtgtggggtc gcgcattttc ttcaaccagg aggtgaggag 60
gtttcgacat ggcggtgcag ccgaaggaga cgctgcagtt ggagagcgcg gccgaggtcg 120
gcttcgtgcg cttctttcag ggcatgccgg agaagccgac caccacagtg cgccttttcg 180
accggggcga cttctatacg gcgcacggcg aggacgcgct gctggccgcc cgggaggtgt 240
tcaagaccca gggggtgatc aagtacatgg ggccggcagg agcaaagaat ctgcagagtg 300
ttgtgcttag taaaatgaat tttgaatctt ttgtaaaaga tcttcttctg gttcgtcagt 360
atagagttga agtttataag aatagagctg gaaataaggc atccaaggag aatgattggt 420
atttggcata taaggcttct cctggcaatc tctctcagtt tgaagacatt ctctttggta 480
acaatgatat gtcagcttcc attggtgttg tgggtgttaa aatgtccgca gttgatggcc 540
agagacaggt tggagttggg tatgtggatt ccatacagag gaaactagga ctgtgtgaat 600
tccctgataa tgatcagttc tccaatcttg aggctctcct catccagatt ggaccaaagg 660
aatgtgtttt acccggagga gagactgctg gagacatggg gaaactgaga cagataattc 720
aaagaggagg aattctgatc acagaaagaa aaaaagctga cttttccaca aaagacattt 780
atcaggacct caaccggttg ttgaaaggca aaaagggaga gcagatgaat agtgctgtat 840
tgccagaaat ggagaatcag gttgcagttt catcactgtc tgcggtaatc aagtttttag 900
aactcttatc agatgattcc aactttggac agtttgaact gactactttt gacttcagcc 960
agtatatgaa attggatatt gcagcagtca gagcccttaa ccttttttcag ggttctgttg 1020
aagataccac tggctctcag tctctggctg ccttgctgaa taagtgtaaa acccctcaag 1080
gacaaagact tgttaaccag tggattaagc agcctctcat ggataagaac agaatagagg 1140
agagattgaa tttagtggaa gcttttgtag aagatgcaga attgaggcag actttacaag 1200

aagatttact tcgtcgattc ccagatctta accgacttgc caagaagttt caaagacaag 1260
cagcaaactt acaagattgt taccgactct atcagggtat aaatcaacta cctaatgtta 1320
tacaggctct ggaaaaacat gaaggaaaac accagaaatt attgttggca gtttttgtga 1380
ctcctcttac tgatcttcgt tctgacttct ccaagtttca ggaaatgata gaaacaactt 1440
tagatatgga tcaggtggaa aaccatgaat tccttgtaaa accttcattt gatcctaatc 1500
tcagtgaatt aagagaaata atgaatgact tggaaaagaa gatgcagtca acattaataa 1560
gtgcagccag agatcttggc ttggaccctg gcaaacagat taaactggat tccagtgcac 1620
agtttggata ttactttcgt gtaacctgta aggaagaaaa agtccttcgt aacaataaaa 1680
actttagtac tgtagatatc cagaagaatg gtgttaaatt taccaacagc aaattgactt 1740
ctttaaatga agagtatacc aaaaataaaa cagaatatga agaagcccag gatgccattg 1800
ttaaagaaat tgtcaatatt tcttcaggct atgtagaacc aatgcagaca ctcaatgatg 1860
tgttagctca gctagatgct gttgtcagct ttgctcacgt gtcaaatgga gcacctgttc 1920
catatgtacg accagccatt ttggagaaag gacaaggaag aattatatta aaagcatcca 1980
ggcatgcttg tgttgaagtt caagatgaaa ttgcatttat tcctaatgac gtatactttg 2040
aaaaagataa acagatgttc cacatcatta ctggccccaa tatgggaggt aaatcaacat 2100
atattcgaca aactggggtg atagtactca tggcccaaat tgggtgtttt gtgccatgtg 2160
agtcagcaga agtgtccatt gtggactgca tcttagcccg agtaggggct ggtgacagtc 2220
aattgaaagg agtctccacg ttcatggctg aaatgttgga aactgcttct atcctcaggt 2280
ctgcaaccaa agattcatta ataatcatag atgaattggg aagaggaact tctacctacg 2340
atggatttgg gttagcatgg gctatatcag aatacattgc aacaaagatt ggtgcttttt 2400
gcatgtttgc aacccatttt catgaactta ctgccttggc caatcagata ccaactgtta 2460
ataatctaca tgtcacagca ctcaccactg aagagacctt aactatgctt tatcaggtga 2520
agaaaggtgt ctgtgatcaa agttttggga ttcatgttgc agagcttgct aatttcccta 2580
agcatgtaat agagtgtgct aaacagaaag ccctggaact tgaggagttt cagtatattg 2640
gagaatcgca aggatatgat atcatggaac cagcagcaaa gaagtgctat ctggaaagag 2700
agcaaggtga aaaaattatt caggagttcc tgtccaaggt gaaacaaatg ccctttactg 2760
aaatgtcaga agaaaacatc acaataaagt taaaacagct aaaagctgaa gtaatagcaa 2820
agaataatag ctttgtaaat gaaatcattt cacgaataaa agttactacg tgaaaaatcc 2880
cagtaatgga atgaaggtaa tattgataag ctattgtctg taatagtttt atattgtttt 2940
atattaaccc ttttccata gtgttaactg tcagtgccca tgggctatca acttaataag 3000
atatttagta atattttact ttgaggacat tttcaaagat ttttattttg aaaaatgaga 3060
gctgtaactg aggactgttt gcaattgaca taggcaataa taagtgatgt gctgaatttt 3120
ataaataaaa tcatgtagtt tgtgg 3145

MLH1 (human) (SEQ ID NO:22)

MSFVAGVIRR LDETVVNRIA AGEVIQRPAN AIKEMIENCL DAKSTSIQVI VKEGGLKLIQ 60
IQDNGTGIRK EDLDIVCERF TTSKLQSFED LASISTYGFR GEALASISHV AHVTITTKTA 120
DGKCAYRASY SDGKLKAPPK PCAGNQGTQI TVEDLFYNIA TRRKALKNPS EEYGKILEVV 180
GRYSVHNAGI SFSVKKQGET VADVRTLPNA STVDNIRSIF GNAVSRELIE IGCEDKTLAF 240
KMNGYISNAN YSVKKCIFLL FINHRLVEST SLRKAIETVY AAYLPKNTHP FLYLSLEISP 300
QNVDVNVHPT KHEVHFLHEE SILERVQQHI ESKLLGSNSS RMYFTQTLLP GLAGPSGEMV 360
KSTTSLTSSS TSGSSDKVYA HQMVRTDSRE QKLDAFLQPL SKPLSSQPQA IVTEDKTDIS 420
SGRARQQDEE MLELPAPAEV AAKNQSLEGD TTKGTSEMSE KRGPTSSNPR KRHREDSDVE 480
MVEDDSRKEM TAACTPRRRI INLTSVLSLQ EEINEQGHEV LREMLHNHSF VGCVNPQWAL 540
AQHQTKLYLL NTTKLSEELF YQILIYDFAN FGVLRLSEPA PLFDLAMLAL DSPESGWTEE 600
DGPKEGLAEY IVEFLKKKAE MLADYFSLEI DEEGNLIGLP LLIDNYVPPL EGLPIFILRL 660
ATEVNWDEEK ECFESLSKEC AMFYSIRKQY ISEESTLSGQ QSEVPGSIPN SWKWTVEHIV 720
YKALRSHILP PKHFTEDGNI LQLANLPDLY KVFERC 756

MLH1 (human) (SEQ ID NO:23)

cttggctctt ctggcgccaa aatgtcgttc gtggcagggg ttattcggcg gctggacgag 60
acagtggtga accgcatcgc ggcgggggaa gttatccagc ggccagctaa tgctatcaaa 120
gagatgattg agaactgttt agatgcaaaa tccacaagta ttcaagtgat tgttaaagag 180
ggaggcctga agttgattca gatccaagac aatggcaccg ggatcaggaa agaagatctg 240
gatattgtat gtgaaaggtt cactactagt aaactgcagt cctttgagga tttagccagt 300
atttctacct atggctttcg aggtgaggct ttggccagca taagccatgt ggctcatgtt 360
actattacaa cgaaaacagc tgatggaaag tgtgcataca gagcaagtta ctcagatgga 420
aaactgaaag cccctcctaa accatgtgct ggcaatcaag ggacccagat cacggtggag 480
gacctttttt acaacatagc cacgaggaga aaagctttaa aaaatccaag tgaagaatat 540
gggaaaattt tggaagttgt tggcaggtat tcagtacaca atgcaggcat tagtttctca 600
gttaaaaaac aaggagagac agtagctgat gttaggacac tacccaatgc ctcaaccgtg 660
gacaatattc gctccatctt tggaaatgct gttagtcgag aactgataga aattggatgt 720
gaggataaaa ccctagcctt caaaatgaat ggttacatat ccaatgcaaa ctactcagtg 780

aagaagtgca tcttcttact cttcatcaac catcgtctgg tagaatcaac ttccttgaga 840
aaagccatag aaacagtgta tgcagcctat ttgcccaaaa acacacaccc attcctgtac 900
ctcagtttag aaatcagtcc ccagaatgtg gatgttaatg tgcaccccac aaagcatgaa 960
gttcacttcc tgcacgagga gagcatcctg gagcgggtgc agcagcacat cgagagcaag 1020
ctcctgggct ccaattcctc caggatgtac ttcacccaga ctttgctacc aggacttgct 1080
ggcccctctg gggagatggt taaatccaca acaagtctga cctcgtcttc tacttctgga 1140
agtagtgata aggtctatgc ccaccagatg gttcgtacag attcccggga acagaagctt 1200
gatgcatttc tgcagcctct gagcaaaccc ctgtccagtc agccccaggc cattgtcaca 1260
gaggataaga cagatatttc tagtggcagg gctaggcagc aagatgagga gatgcttgaa 1320
ctcccagccc ctgctgaagt ggctgccaaa aatcagagct tggaggggga tacaacaaag 1380
gggacttcag aaatgtcaga gaagagagga cctacttcca gcaaccccag aaagagacat 1440
cgggaagatt ctgatgtgga aatggtggaa gatgattccc gaaaggaaat gactgcagct 1500
tgtacccccc ggagaaggat cattaacctc actagtgttt tgagtctcca ggaagaaatt 1560
aatgagcagg gacatgaggt tctccgggag atgttgcata accactcctt cgtgggctgt 1620
gtgaatcctc agtgggcctt ggcacagcat caaaccaagt tataccttct caacaccacc 1680
aagcttagtg aagaactgtt ctaccagata ctcatttatg attttgccaa ttttggtgtt 1740
ctcaggttat cggagccagc accgctcttt gaccttgcca tgcttgcctt agatagtcca 1800
gagagtggct ggacagagga agatggtccc aaagaaggac ttgctgaata cattgttgag 1860
tttctgaaga agaaggctga gatgcttgca gactatttct ctttggaaat tgatgaggaa 1920
gggaacctga ttggattacc ccttctgatt gacaactatg tgccccttt ggagggactg 1980
cctatcttca ttcttcgact agccactgag gtgaattggg acgaagaaaa ggaatgtttt 2040
gaaagcctca gtaaagaatg cgctatgttc tattccatcc ggaagcagta catatctgag 2100
gagtcgaccc tctcaggcca gcagagtgaa gtgcctggct ccattccaaa ctcctggaag 2160
tggactgtgg aacacattgt ctataaagcc ttgcgctcac acattctgcc tcctaaacat 2220
ttcacagaag atggaaatat cctgcagctt gctaacctgc ctgatctata caaagtcttt 2280
gagaggtgtt aaatatggtt atttatgcac tgtgggatgt gttcttcttt ctctgtattc 2340
cgatacaaag tgttgtatca aagtgtgata tacaaagtgt accaacataa gtgttggtag 2400
cacttaagac ttatacttgc cttctgatag tattccttta tacacagtgg attgattata 2460
aataaataga tgtgtcttaa cata 2484

hPMS2-134 (human) (SEQ ID NO:24)

MERAESSSTE PAKAIKPIDR KSVHQICSGQ VVLSLSTAVK ELVENSLDAG ATNIDLKLKD 60
YGVDLIEVSD NGCGVEEENF EGLTLKHHTS KIQEFADLTQ VETFGFRGEA LSSLCALSDV 120
TISTCHASAK VGT 133

hPMS2-134 (human cDNA) (SEQ ID NO:25)

cgaggcggat cgggtgttgc atccatggag cgagctgaga gctcgagtac agaacctgct 60
aaggccatca aacctattga tcggaagtca gtccatcaga tttgctctgg gcaggtggta 120
ctgagtctaa gcactgcggt aaaggagtta gtagaaaaca gtctggatgc tggtgccact 180
aatattgatc taaagcttaa ggactatgga gtggatctta ttgaagtttc agacaatgga 240
tgtggggtag aagaagaaaa cttcgaaggc ttaactctga aacatcacac atctaagatt 300
caagagtttg ccgacctaac tcaggttgaa acttttggct ttcgggggga agctctgagc 360
tcactttgtg cactgagcga tgtcaccatt tctacctgcc acgcatcggc gaaggttgga 420
acttga 426

GTBP (human) (SEQ ID NO:26)

MSRQSTLYSF FPKSPALSDA NKASARASRE GGRAAAAPGA SPSPGGDAAW SEAGPGPRPL 60
ARSASPPKAK NLNGGLRRSV APAAPTSCDF SPGDLVWAKM EGYPWWPCLV YNHPFDGTFI 120
REKGKSVRVH VQFFDDSPTR GWVSKRLLKP YTGSKSKEAQ KGGHFYSAKP EILRAMQRAD 180
EALNKDKIKR LELAVCDEPS EPEEEEEMEV GTTYVTDKSE EDNEIESEEE VQPKTQGSRR 240
SSRQIKKRRV ISDSESDIGG SDVEFKPDTK EEGSSDEISS GVGDSESEGL NSPVKVARKR 300
KRMVTGNGSL KRKSSRKETP SATKQATSIS SETKNTLRAF SAPQNSESQA HVSGGGDDSS 360
RPTVWYHETL EWLKEEKRRD EHRRRPDHPD FDASTLYVPE DFLNSCTPGM RKWWQIKSQN 420
FDLVICYKVG KFYELYHMDA LIGVSELGLV FMKGNWAHSG FPEIAFGRYS DSLVQKGYKV 480
ARVEQTETPE MMEARCRKMA HISKYDRVVR REICRIITKG TQTYSVLEGD PSENYSKYLL 540
SLKEKEEDSS GHTRAYGVCF VDTSLGKFFI GQFSDDRHCS RFRTLVAHYP PVQVLFEKGN 600
LSKETKTILK SSLSCSLQEG LIPGSQFWDA SKTLRTLLEE EYFREKLSDG IGVMLPQVLK 660
GMTSESDSIG LTPGEKSELA LSALGGCVFY LKKCLIDQEL LSMANFEEYI PLDSDTVSTT 720
RSGAIFTKAY QRMVLDAVTL NNLEIFLNGT NGSTEGTLLE RVDTCHTPFG KRLLKQWLCA 780
PLCNHYAIND RLDAIEDLMV VPDKISEVVE LLKKLPDLER LLSKIHNVGS PLKSQNHPDS 840
RAIMYEETTY SKKKIIDFLS ALEGFKVMCK IIGIMEEVAD GFKSKILKQV ISLQTKNPEG 900

RFPDLTVELN RWDTAFDHEK ARKTGLITPK AGFDSDYDQA LADIRENEQS LLEYLEKQRN 960
RIGCRTIVYW GIGRNRYQLE IPENFTTRNL PEEYELKSTK KGCKRYWTKT IEKKLANLIN 1020
AEERRDVSLK DCMRRLFYNF DKNYKDWQSA VECIAVLDVL LCLANYSRGG DGPMCRPVIL 1080
LPEDTPPFLE LKGSRHPCIT KTFFGDDFIP NDILIGCEEE EQENGKAYCV LVTGPNMGGK 1140
STLMRQAGLL AVMAQMGCYV PAEVCRLTPI DRVFTRLGAS DRIMSGESTF FVELSETASI 1200
LMHATAHSLV LVDELGRGTA TFDGTAIANA VVKELAETIK CRTLFSTHYH SLVEDYSQNV 1260
AVRLGHMACM VENECEDPSQ ETITFLYKFI KGACPKSYGF NAARLANLPE EVIQKGHRKA 1320
REFEKMNQSL RLFREVCLAS ERSTVDAEAV HKLLTLIKEL 1360

GTBP (human cDNA) (SEQ ID NO:27)

gccgcgcggt agatgcggtg cttttaggag ctccgtccga cagaacggtt gggccttgcc 60
ggctgtcggt atgtcgcgac agagcaccct gtacagcttc ttccccaagt ctccggcgct 120
gagtgatgcc aacaaggcct cggccagggc ctcacgcgaa ggcggccgtg ccgccgctgc 180
ccccggggcc tctccttccc caggcgggga tgcggcctgg agcgaggctg ggcctgggcc 240
caggcccttg gcgcgctccg cgtcaccgcc caaggcgaag aacctcaacg gagggctgcg 300
gagatcggta gcgcctgctg cccccaccag ttgtgacttc tcaccaggag atttggtttg 360
ggccaagatg gagggttacc cctggtggcc ttgtctggtt tacaaccacc cctttgatgg 420
aacattcatc cgcgagaaag ggaaatcagt ccgtgttcat gtacagtttt ttgatgacag 480
cccaacaagg ggctgggtta gcaaaaggct tttaaagcca tatacaggtt caaaatcaaa 540
ggaagcccag aagggaggtc atttttacag tgcaaagcct gaaatactga gagcaatgca 600
acgtgcagat gaagccttaa ataaagacaa gattaagagg cttgaattgg cagtttgtga 660
tgagccctca gagccagaag aggaagaaga gatggaggta ggcacaactt acgtaacaga 720
taagagtgaa gaagataatg aaattgagag tgaagaggaa gtacagccta agacacaagg 780
atctaggcga agtagccgcc aaataaaaaa acgaagggtc atatcagatt ctgagagtga 840
cattggtggc tctgatgtgg aatttaagcc agacactaag gaggaaggaa gcagtgatga 900
aataagcagt ggagtggggg atagtgagag tgaaggcctg aacagccctg tcaaagttgc 960
tcgaaagcgg aagagaatgg tgactggaaa tggctctctt aaaaggaaaa gctctaggaa 1020
ggaaacgccc tcagccacca aacaagcaac tagcatttca tcagaaacca agaatacttt 1080
gagagctttc tctgcccctc aaaattctga atcccaagcc cacgttagtg gaggtggtga 1140
tgacagtagt cgccctactg tttggtatca tgaaacttta gaatggctta aggaggaaaa 1200
gagaagagat gagcacagga ggaggcctga tcaccccgat tttgatgcat ctacactcta 1260
tgtgcctgag gatttcctca attcttgtac tcctgggatg aggaagtggt ggcagattaa 1320
gtctcagaac tttgatcttg tcatctgtta caaggtgggg aaattttatg agctgtacca 1380
catggatgct cttattggag tcagtgaact ggggctggta ttcatgaaag gcaactgggc 1440
ccattctggc tttcctgaaa ttgcatttgg ccgttattca gattccctgg tgcagaaggg 1500
ctataaagta gcacgagtgg aacagactga gactccagaa atgatggagg cacgatgtag 1560
aaagatggca catatatcca agtatgatag agtggtgagg agggagatct gtaggatcat 1620
taccaagggt acacagactt acagtgtgct ggaaggtgat ccctctgaga actacagtaa 1680
gtatcttctt agcctcaaag aaaaagagga agattcttct ggccatactc gtgcatatgg 1740
tgtgtgcttt gttgatactt cactgggaaa gtttttcata ggtcagtttt cagatgatcg 1800
ccattgttcg agatttagga ctctagtggc acactatccc ccagtacaag ttttatttga 1860
aaaaggaaat ctctcaaagg aaactaaaac aattctaaag agttcattgt cctgttctct 1920
tcaggaaggt ctgataccg gctcccagtt ttgggatgca tccaaaactt tgagaactct 1980
ccttgaggaa gaatatttta gggaaaagct aagtgatggc attgggggtga tgttacccca 2040
ggtgcttaaa ggtatgactt cagagtctga ttccattggg ttgacaccag gagagaaaag 2100
tgaattggcc ctctctgctc taggtggttg tgtcttctac ctcaaaaaat gccttattga 2160
tcaggagctt ttatcaatgg ctaattttga agaatatatt cccttggatt ctgacacagt 2220
cagcactaca agatctggtg ctatcttcac caaagcctat caacgaatgg tgctagatgc 2280
agtgacatta aacaacttgg agatttttct gaatggaaca aatggttcta ctgaaggaac 2340
cctactagag agggttgata cttgccatac tccttttggt aagcggctcc taaagcaatg 2400
gctttgtgcc ccactctgta accattatgc tattaatgat cgtctagatg ccatagaaga 2460
cctcatggtt gtgcctgaca aaatctccga agttgtagag cttctaaaga agcttccaga 2520
tcttgagagg ctactcagta aaattcataa tgttgggtct cccctgaaga gtcagaacca 2580
cccagacagc agggctataa tgtatgaaga aactacatac agcaagaaga agattattga 2640
ttttctttct gctctggaag gattcaaagt aatgtgtaaa attatagga tcatggaaga 2700
agttgctgat ggttttaagt ctaaaatcct taagcaggtc atctctctgc agacaaaaaa 2760
tcctgaaggt cgttttcctg atttgactgt agaattgaac cgatgggata cagcctttga 2820
ccatgaaaag gctcgaaaga ctggacttat tactcccaaa gcaggctttg actctgatta 2880
tgaccaagct cttgctgaca taagagaaaa tgaacagagc ctcctggaat acctagagaa 2940
acagcgcaac agaattggct gtaggaccat agtctattgg gggattggta ggaaccgtta 3000
ccagctggaa attcctgaga atttcaccac tcgcaatttg ccagaagaat acgagttgaa 3060
atctaccaag aagggctgta aacgatactg gaccaaaact attgaaaaga agttggctaa 3120
tctcataaat gctgaagaac ggagggatgt atcattgaag gactgcatgc ggcgactgtt 3180
ctataacttt gataaaaatt acaaggactg gcagtctgct gtagagtgta tcgcagtgtt 3240

```
ggatgtttta ctgtgcctgg ctaactatag tcgagggggt gatggtccta tgtgtcgccc 3300
agtaattctg ttgccggaag atacccccgc cttcttagag cttaaaggat cacgccatcc 3360
ttgcattacg aagacttttt ttggagatga ttttattcct aatgacattc taataggctg 3420
tgaggaagag gagcaggaaa atggcaaagc ctattgtgtg cttgttactg gaccaaatat 3480
ggggggcaag tctacgctta tgagacaggc tggcttatta gctgtaatgg cccagatggg 3540
ttgttacgtc cctgctgaag tgtgcaggct cacaccaatt gatagagtgt ttactagact 3600
tggtgcctca gacagaataa tgtcaggtga aagtacattt tttgttgaat taagtgaaac 3660
tgccagcata ctcatgcatg caacagcaca ttctctggtg cttgtggatg aattaggaag 3720
aggtactgca acatttgatg ggacggcaat agcaaatgca gttgttaaag aacttgctga 3780
gactataaaa tgtcgtacat tattttcaac tcactaccat tcattagtag aagattattc 3840
tcaaaatgtt gctgtgcgcc taggacatat ggcatgcatg gtagaaaatg aatgtgaaga 3900
ccccagccag gagactatta cgttcctcta taaattcatt aagggagctt gtcctaaaag 3960
ctatggcttt aatgcagcaa ggcttgctaa tctcccagag gaagttattc aaaagggaca 4020
tagaaaagca agagaatttg agaagatgaa tcagtcacta cgattatttc gggaagtttg 4080
cctggctagt gaaaggtcaa ctgtagatgc tgaagctgtc cataaattgc tgactttgat 4140
taaggaatta tagactgact acattggaag ctttgagttg acttctgaca aaggtggtaa 4200
attcagacaa cattatgatc taataaactt tatttttaa aaat                   4244
```

MSH3 (human) (SEQ ID NO:28)

```
MSRRKPASGG LAASSSAPAR QAVLSRFFQS TGSLKSTSSS TGAADQVDPG AAAAAAPPAP 60
AFPPQLPPHV ATEIDRRKKR PLENDGPVKK KVKKVQQKEG GSDLGMSGNS EPKKCLRTRN 120
VSKSLEKLKE FCCDSALPQS RVQTESLQER FAVLPKCTDF DDISLLHAKN AVSSEDSKRQ 180
INQKDTTLFD LSQFGSSNTS HENLQKTASK SANKRSKSIY TPLELQYIEM KQQHKDAVLC 240
VECGYKYRFF GEDAEIAARE LNIYCHLDHN FMTASIPTHR LFVHVRRLVA KGYKVGVVKQ 300
TETAALKAIG DNRSSLFSRK LTALYTKSTL IGEDVNPLIK LDDAVNVDEI MTDTSTSYLL 360
CISENKENVR DKKKGNIFIG IVGVQPATGE VVFDSFQDSA SRSELETRMS SLQPVELLLP 420
SALSEQTEAL IHRATSVSVQ DDRIRVERMD NIYFEYSHAF QAVTEFYAKD TVDIKGSQII 480
SGIVNLEKPV ICSLAAIIKY LKEFNLEKML SKPENFKQLS SKMEFMTING TTLRNLEILQ 540
NQTDMKTKGS LLWVLDHTKT SFGRRKLKKW VTQPLLKLRE INARLDAVSE VLHSESSVFG 600
QIENHLRKLP DIERGLCSIY HKKCSTQEFF LIVKTLYHLK SEFQAIIPAV NSHIQSDLLR 660
TVILEIPELL SPVEHYLKIL NEQAAKVGDK TELFKDLSDF PLIKKRKDEI QGVIDEIRMH 720
LQEIRKILKN PSAQYVTVSG QEFMIEIKNS AVSCIPTDWV KVGSTKAVSR FHSPFIVENY 780
RHLNQLREQL VLDCSAEWLD FLEKFSEHYH SLCKAVHHLA TVDCIFSLAK VAKQGDYCRP 840
TVQEERKIVI KNGRHPVIDV LLGEQDQYVP NNTDLSEDSE RVMIITGPNM GGKSSYIKQV 900
ALITIMAQIG SYVPAEEATI GIVDGIFTRM GAADNIYKGR STFMEELTDT AEIIRKATSQ 960
SLVILDELGR GTSTHDGIAI AYATLEYFIR DVKSLTLFVT HYPPVCELEK NYSHQVGNYH 1020
MGFLVSEDES KLDPGAAEQV PDFVTFLYQI TRGIAARSYG LNVAKLADVP GEILKKAAHK 1080
SKELEGLINT KRKRLKYFAK LWTMHNAQDL QKWTEEFNME ETQTSLLH             1128
```

MSH3 (human DNA) (SEQ ID NO:29)

```
gggcacgagc cctgccatgt ctcgccggaa gcctgcgtcg ggcggcctcg ctgcctccag 60
ctcagcccct gcgaggcaag cggttttgag ccgattcttc cagtctacgg gaagcctgaa 120
atccacctcc tcctccacag gtgcagccga ccaggtggac cctggcgctg cagcggccgc 180
agcgccccca gcgcccgcct tcccgcccca gctgccgccg cacgtagcta cagaaattga 240
cagaagaaag aagagaccat tggaaaatga tgggcctgtt aaaaagaaag taaagaaagt 300
ccaacaaaag gaaggaggaa gtgatctggg aatgtctggc aactctgagc caaagaaatg 360
tctgaggacc aggaatgttt caaagtctct ggaaaaattg aaagaattct gctgcgattc 420
tgcccttcct caaagtagag tccagacaga atctctgcag gagagatttg cagttctgcc 480
aaaatgtact gattttgatg atatcagtct tctacacgca aagaatgcag tttcttctga 540
agattcgaaa cgtcaaatta atcaaaagga cacaacactt tttgatctca gtcagtttgg 600
atcatcaaat acaagtcatg aaaatttaca gaaaactgct tccaaatcag ctaacaaacg 660
gtccaaaagc atctatacgc cgctagaatt acaatacata gaaatgaagc agcagcacaa 720
agatgcagtt ttgtgtgtgg aatgtggata taagtataga ttctttgggg aagatgcaga 780
gattgcagcc cgagagctca atatttattg ccatttagat cacaacttta tgacagcaag 840
tatacctact cacagactgt ttgttcatgt acgccgcctg gtggcaaaag gatataaggt 900
gggagttgtg aagcaaactg aaactgcagc attaaaggcc attggagaca acagaagttc 960
actcttttcc cggaaattga ctgcccttta tacaaaatct acacttattg gagaagatgt 1020
gaatccccta atcaagctgg atgatgctgt aaatgttgat gagataatga ctgatacttc 1080
taccagctat cttctgtgca tctctgaaaa taaggaaaat gttagggaca aaaaaaaggg 1140
caacattttt attggcattg tgggagtgca gcctgccaca ggcgaggttg tgtttgatag 1200
tttccaggac tctgcttctc gttcagagct agaaacccgg atgtcaagcc tgcagccagt 1260
agagctgctg cttccttcgg ccttgtccga gcaaacagag gcgctcatcc acagagccac 1320
atctgttagt gtgcaggatg acagaattcg agtcgaaagg atggataaca tttattttga 1380
```

atacagccat gctttccagg cagttacaga gttttatgca aaagatacag ttgacatcaa 1440
aggttctcaa attatttctg gcattgttaa cttagagaag cctgtgattt gctctttggc 1500
tgccatcata aaatacctca aagaattcaa cttggaaaag atgctctcca aacctgagaa 1560
ttttaaacag ctatcaagta aaatggaatt tatgacaatt aatggaacaa cattaaggaa 1620
tctggaaatc ctacagaatc agactgatat gaaaaccaaa ggaagtttgc tgtgggtttt 1680
agaccacact aaaacttcat ttgggagacg gaagttaaag aagtgggtga cccagccact 1740
ccttaaatta agggaaataa atgcccggct tgatgctgta tcggaagttc tccattcaga 1800
atctagtgtg tttggtcaga tagaaaatca tctacgtaaa ttgcccgaca tagagagggg 1860
actctgtagc atttatcaca aaaaatgttc tacccaagag ttcttcttga ttgtcaaaac 1920
tttatatcac ctaaagtcag aatttcaagc aataatacct gctgttaatt cccacattca 1980
gtcagacttg ctccggaccg ttattttaga aattcctgaa ctcctcagtc cagtggagca 2040
ttacttaaag atactcaatg aacaagctgc caaagttggg gataaaactg aattatttaa 2100
agacctttct gacttccctt taataaaaaa gaggaaggat gaaattcaag gtgttattga 2160
cgagatccga atgcatttgc aagaaatacg aaaaatacta aaaaatcctt ctgcacaata 2220
tgtgacagta tcaggacagg agtttatgat agaaataaag aactctgctg tatcttgtat 2280
accaactgat tgggtaaagg ttggaagcac aaaagctgtg agccgctttc actctccttt 2340
tattgtagaa aattacagac atctgaatca gctccgggag cagctagtcc ttgactgcag 2400
tgctgaatgg cttgattttc tagagaaatt cagtgaacat tatcactcct tgtgtaaagc 2460
agtgcatcac ctagcaactg ttgactgcat tttctccctg gccaaggtcg ctaagcaagg 2520
agattactgc agaccaactg tacaagaaga aagaaaaatt gtaataaaaa atggaaggca 2580
ccctgtgatt gatgtgttgc tgggagaaca ggatcaatat gtcccaaata atacagattt 2640
atcagaggac tcagagagag taatgataat taccggacca aacatgggtg gaaagagctc 2700
ctacataaaa caagttgcat tgattaccat catggctcag attggctcct atgttcctgc 2760
agaagaagcg acaattggga ttgtggatgg cattttcaca aggatgggtg ctgcagacaa 2820
tatatataaa ggacggagta catttatgga agaactgact gacacagcag aaataatcag 2880
aaaagcaaca tcacagtcct tggttatctt ggatgaacta ggaagaggga cgagcactca 2940
tgatggaatt gccattgcct atgctacact tgagtatttc atcagagatg tgaaatcctt 3000
aaccctgttt gtcacccatt atccgccagt ttgtgaacta gaaaaaaatt actcacacca 3060
ggtggggaat taccacatgg gattcttggt cagtgaggat gaaagcaaac tggatccagg 3120
cgcagcagaa caagtccctg attttgtcac cttcctttac caaataacta gaggaattgc 3180
agcaaggagt tatggattaa atgtggctaa actagcagat gttcctggag aaattttgaa 3240
gaaagcagct cacaagtcaa aagagctgga aggattaata aatacgaaaa gaaagagact 3300
caagtatttt gcaaagttat ggacgatgca taatgcacaa gacctgcaga agtggacaga 3360
ggagttcaac atggaagaaa cacagacttc tcttcttcat taaaatgaag actacatttg 3420
tgaacaaaaa atggagaatt aaaaatacca actgtacaaa ataactctcc agtaacagcc 3480
tatctttgtg tgacatgtga gcataaaatt atgaccatgg tatattccta ttggaaacag 3540
agaggttttt ctgaagacag tctttttcaa gtttctgtct tcctaacttt tctacgtata 3600
aacactcttg aatagacttc cactttgtaa ttagaaaatt ttatggacag taagtccagt 3660
aaagccttaa gtggcagaat ataattccca agcttttgga gggtgatata aaaatttact 3720
tgatattttt atttgtttca gttcagataa ttggcaactg ggtgaatctg gcaggaatct 3780
atccattgaa ctaaaataat tttattatgc aaccagttta tccaccaaga acataagaat 3840
tttttataag tagaaagaat tggccaggca tggtggctca tgcctgtaat cccagcactt 3900
tgggaggcca aggtaggcag atcacctgag gtcaggagtt caagaccagc ctggccaaca 3960
tggcaaaacc ccatctttac taaaaatata aagtacatct ctactaaaaa tacgaaaaaa 4020
ttagctgggc atggtggcgc acacctgtag tcccagctac tccggaggct gaggcaggag 4080
aatctcttga acctgggagg cggaggttgc aatgagccga gatcacgtca ctgcactcca 4140
gcttgggcaa cagagcaaga ctccatctca aaaaagaaaa aagaaaagaa atagaattat 4200
caagctttta aaaactagag cacagaagga ataaggtcat gaaatttaaa aggttaaata 4260
ttgtcatagg attaagcagt ttaaagattg ttggatgaaa ttatttgtca ttcattcaag 4320
taataaatat ttaatgaata cttgctataa aaaaaaaaaa aaaaaaaaaa aaaa 4374

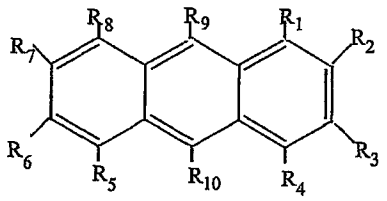
Each reference cited herein is hereby incorporated by reference in its entirety.

We claim:

1. A method for making a hypermutable cell comprising exposing a cell to an inhibitor of mismatch repair, wherein said inhibitor is an anthracene, an ATPase inhibitor, a nuclease inhibitor, a polymerase inhibitor, or an antisense oligonucleotide that specifically hybridizes to a nucleotide encoding a mismatch repair protein.

2. The method of claim 1 wherein said inhibitor is an anthracene.

3. The method of claim 2 wherein said anthracene has the formula:



wherein R_1-R_{10} are independently hydrogen, hydroxyl, amino, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl,O-alkynyl, S-alkynyl, N-alkynyl, aryl, substituted aryl, aryloxy, substituted aryloxy, heteroaryl, substituted heteroaryl, aralkyloxy, arylalkyl, alkylaryl, alkylaryloxy, arylsulfonyl, alkylsulfonyl, alkoxycarbonyl, aryloxycarbonyl, guanidino, carboxy, an alcohol, an amino acid, sulfonate, alkyl sulfonate, CN, NO_2, an aldehyde group, an ester, an ether, a crown ether, a ketone, an organosulfur compound, an organometallic group, a carboxylic acid, an organosilicon or a carbohydrate that optionally contains one or more alkylated hydroxyl groups;

wherein said heteroalkyl, heteroaryl, and substituted heteroaryl contain at least one heteroatom that is oxygen, sulfur, a metal atom, phosphorus, silicon or nitrogen; and

wherein said substituents of said substituted alkyl, substituted alkenyl, substituted alkynyl,

substituted aryl, and substituted heteroaryl are halogen, CN, NO_2, lower alkyl, aryl, heteroaryl, aralkyl, aralkyloxy, guanidino, alkoxycarbonyl, alkoxy, hydroxy, carboxy and amino;

and wherein said amino groups optionally substituted with an acyl group, or 1 to 3 aryl or lower alkyl groups.

4. The method of claim 3 wherein R_5 and R_6 are hydrogen.

5. The method of claim 3 wherein R_1-R_{10} are independently hydrogen, hydroxyl, alkyl, aryl, arylaklyl, or hydroxyalkyl.

6. The method of claim 3 wherein R_1-R_{10} are independently hydrogen, hydroxyl, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, phenyl, tolyl, hydroxymethyl, hydroxypropyl, or hydroxybutyl.

7. The method of claim 3 wherein said anthracene is selected from the group consisting of 1,2-dimethylanthracene, 9,10-dimethyl anthracene, 7,8-dimethylanthracene, 9,10-diphenylanthracene, 9,10-dihydroxymethylanthracene, 9-hydroxymethyl-10-methylanthracene, dimethylanthracene-1,2-diol, 9-hydroxymethyl-10-methylanthracene-1,2-diol, 9-hydroxymethyl-10-methylanthracene-3,4-diol, and 9, 10-di-m-tolyanthracene.

8. The method of claim 3 wherein R_3, R_4, R_5, R_6, R_7, R_8, R_9 and R_{10} are hydrogen.

9. The method of claim 3 wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7 and R_8 are hydrogen.

10. The method of claim 3 wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7 and R_8 are hydrogen.

11. The method of claim 3 wherein R_1, R_2, R_3, R_4, R_5, R_6, R_9 and R_{10} are hydrogen.

12. The method of claim 3 wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7 and R_8 are hydrogen.

13. The method of claim 3 wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 and R_{10} are hydrogen.

14. The method of claim 1 wherein said ATPase inhibitor is nonhydrolyzable forms of ATP such as AMP-PNP.

15. The method of claim 1 wherein said a nuclease inhibitor is an analog of N-

Ethylmaleimide, a heterodimeric adenine-chain-acridine compounds, or a quinilone such as Heliquinomycin.

16. The method of claim 1 wherein said polymerase inhibitor is an analog of aphidicolin,1-(2'-Deoxy-2'-fluoro-beta-L-arabinofuranosyl)-5-methyluracil (L-FMAU) or 2',3'-dideoxyribonucleoside 5'-triphosphates.

17. The method of claim 1 wherein said antisense oligonucleotide comprises about 15 consecutive nucleotides that are complementary to the coding strand of a mismatch repair protein, wherein said antisense oligonucleotide specifically binds to said coding strand of said mismatch repair protein under physiological conditions and inhibits mismatch repair activity of said mismatch repair protein.

18. The method of claim 17 wherein said antisense oligonucleotide specifically binds to a regulatory portion on said coding strand of said mismatch repair protein.

19. The method of claim 17 wherein said antisense oligonucleotide is directed against the first six codons of a MMR gene message.

20. The method of claim 1 wherein said inhibitor of mismatch repair is introduced into a growth medium of a eukaryotic cell *in vitro*.

21. The method of claim 1 wherein said inhibitor of mismatch repair is introduced into a growth medium of a prokaryotic cell *in vitro*.

22. The method of claim 1 wherein said inhibitor of mismatch repair is introduced into a growth medium of a plant.

23. A method for generating a mutation in a gene of interest comprising exposing a cell comprising said gene of interest to a chemical mismatch repair inhibitor and testing said cell to determine whether said gene of interest comprises a mutation.

24. The method of claim 23 wherein said testing comprises analyzing a polynucleotide sequence of said gene of interest.

25. The method of claim 23 wherein said testing comprises analyzing a protein encoded by said gene of interest.

26. The method of claim 23 wherein said testing comprises analyzing the phenotype of said cell.

27. The method of claim 23 wherein said cell is a mammalian cell, and wherein said mammalian cell is made mismatch repair defective by exposing said mammalian cell to an inhibitor of mismatch repair.

28. The method of claim 27 further comprising removing the chemical inhibitor of mismatch repair after determining that said gene of interest comprises a mutation.

29. The method of claim 27 wherein said testing comprises analyzing a polynucleotide sequence of said gene of interest.

30. The method of claim 27 wherein said testing comprises analyzing a protein encoded by said gene of interest.

31. The method of claim 27 wherein said testing comprises analyzing the phenotype of said cell.

32. A method for generating a mutation in a gene of interest comprising exposing an animal to a chemical inhibitor of mismatch repair and testing said animal to determine whether the gene of interest comprises a mutation.

33. The method of claim 32 wherein said animal is a mammal.

34. The method of claim 32 wherein said testing comprises analyzing a polynucleotide sequence of said gene of interest.

35. The method of claim 32 wherein said testing comprises analyzing a protein encoded by said gene of interest.

36. The method of claim 32 wherein said testing comprises analyzing the phenotype of said cell.

37. The method of claim 33 wherein said mammal is made mismatch repair defective by exposing said mammal to an inhibitor of mismatch repair.

38. The method of claim 37 further comprising removing said inhibitor of mismatch repair after determining that said gene of interest comprises a mutation.

39. A hypermutable transgenic mammal made by the method of claim 33.

40. A method for generating a mismatch repair defective plant comprising exposing said plant to an inhibitor of mismatch repair.

41. A method for generating a mutation in a gene of interest comprising growing a plant comprising said gene of interest, exposing said plant to an inhibitor of mismatch repair, and testing said plant to determine whether said gene of interest comprises a mutation.

42. The method of claim 41 wherein said testing comprises analyzing a polynucleotide sequence of said gene of interest.

43. The method of claim 41 wherein said testing comprises analyzing a protein encoded by said gene of interest.

44. The method of claim 41 wherein said testing comprises analyzing the phenotype of

said plant.

45. The method of claim 41 wherein said plant is made mismatch repair defective by exposing said plant to an inhibitor of mismatch repair.

46. A hypermutable plant made by the method of claim 40.

47. The plant of claim 46 wherein said plant is monocot.

48. The plant of claim 46 wherein said plant is dicot.

49. A method for screening for chemical inhibitors of mismatch repair comprising exposing an organism to a candidate compound and screening the DNA of said organism for microsatellite instability.

50. The method of claim 49 wherein said organism is a mammal.

51. The method of claim 49 wherein said organism is a microbe.

52. The method of claim 49 wherein said organism is a plant.

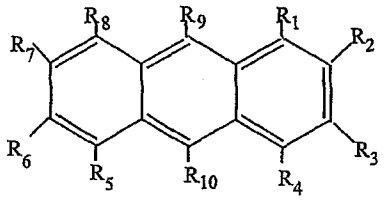
53. The method of claim 49 wherein said screening comprises monitoring endogenous microsatellites.

54. The method of claim 49 wherein said screening comprises the use of reporter expression genes, wherein said reporter expression genes comprise polynucleotide repeats within a coding region of said reporter gene.

55. The method of claim 54 wherein said reporter gene is β-glucuronidase.

56. A method for blocking mismatch repair activity *in vivo* comprising exposing a cell to an anthracene compound.

57. The method of claim 56 wherein said anthracene comprises the formula:



wherein R_1-R_{10} are independently hydrogen, hydroxyl, amino, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl,O-alkynyl, S-alkynyl, N-alkynyl, aryl, substituted aryl, aryloxy, substituted aryloxy, heteroaryl, substituted heteroaryl, aralkyloxy, arylalkyl, alkylaryl, alkylaryloxy, arylsulfonyl, alkylsulfonyl, alkoxycarbonyl, aryloxycarbonyl, guanidino, carboxy, an alcohol, an amino acid, sulfonate, alkyl sulfonate, CN, NO_2, an aldehyde group, an ester, an ether, a crown ether, a ketone, an organosulfur compound, an organometallic group, a carboxylic acid, an organosilicon or a carbohydrate that optionally contains one or more alkylated hydroxyl groups;

wherein said heteroalkyl, heteroaryl, and substituted heteroaryl contain at least one heteroatom that is oxygen, sulfur, a metal atom, phosphorus, silicon or nitrogen; and

wherein said substituents of said substituted alkyl, substituted alkenyl, substituted alkynyl,
substituted aryl, and substituted heteroaryl are halogen, CN, NO_2, lower alkyl, aryl, heteroaryl, aralkyl, aralkyloxy, guanidino, alkoxycarbonyl, alkoxy, hydroxy, carboxy and amino;

and wherein said amino groups optionally substituted with an acyl group, or 1 to 3 aryl or lower alkyl groups.

58. The method of claim 57 wherein R_5 and R_6 are hydrogen.

59. The method of claim 57 wherein R_1-R_{10} are independently hydrogen, hydroxyl, alkyl, aryl, arylaklyl, or hydroxyalkyl.

60. The method of claim 57 wherein R_1-R_{10} are independently hydrogen, hydroxyl, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, phenyl, tolyl, hydroxymethyl, hydroxypropyl, or hydroxybutyl.

61. The method of claim 57 wherein said anthracene is selected from the group consisting of 1,2-dimethylanthracene, 9,10-dimethyl anthracene, 7,8-dimethylanthracene, 9,10-diphenylanthracene, 9,10-dihydroxymethylanthracene, 9-hydroxymethyl-10-methylanthracene, dimethylanthracene-1,2-diol, 9-hydroxymethyl-10-methylanthracene-1,2-diol, 9-hydroxymethyl-10-methylanthracene-3,4-diol, and 9, 10-di-m-tolyanthracene.
R_3, R_4,

62. The method of claim 57 wherein R_3, R_4, R_5, R_6, R_7, R_8, R_9 and R_{10} are hydrogen.

63. The method of claim 57 wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7 and R_8 are hydrogen.

64. The method of claim 57 wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7 and R_8 are hydrogen.

65. The method of claim 57 wherein R_1, R_2, R_3, R_4, R_5, R_6, R_9 and R_{10} are hydrogen.

66. The method of claim 57 wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7 and R_8 are hydrogen.

67. The method of claim 57 wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 and R_{10} are hydrogen.

68. The method of claim 23 further comprising exposing said cell to a mutagen.

69. The method of claim 32 further comprising exposing said animal to a mutagen.

70. The method of claim 68 or 69 wherein said mutagen is selected from the group consisting of N-methyl-N'-nitro-N-nitrosoguanidine, methane sulfonate, dimethyl sulfonate, O-6-methyl benzadine, ethyl methanesulfonate, methylnitrosourea, and ethylnitrosourea.

71. The method of claim 49 wherein the chemcial is a MMR inhibitor wherein it induces microsatellite instability in MMR proficient cells but does not induce enhanced microsattelite instability in MMR deficient cells.

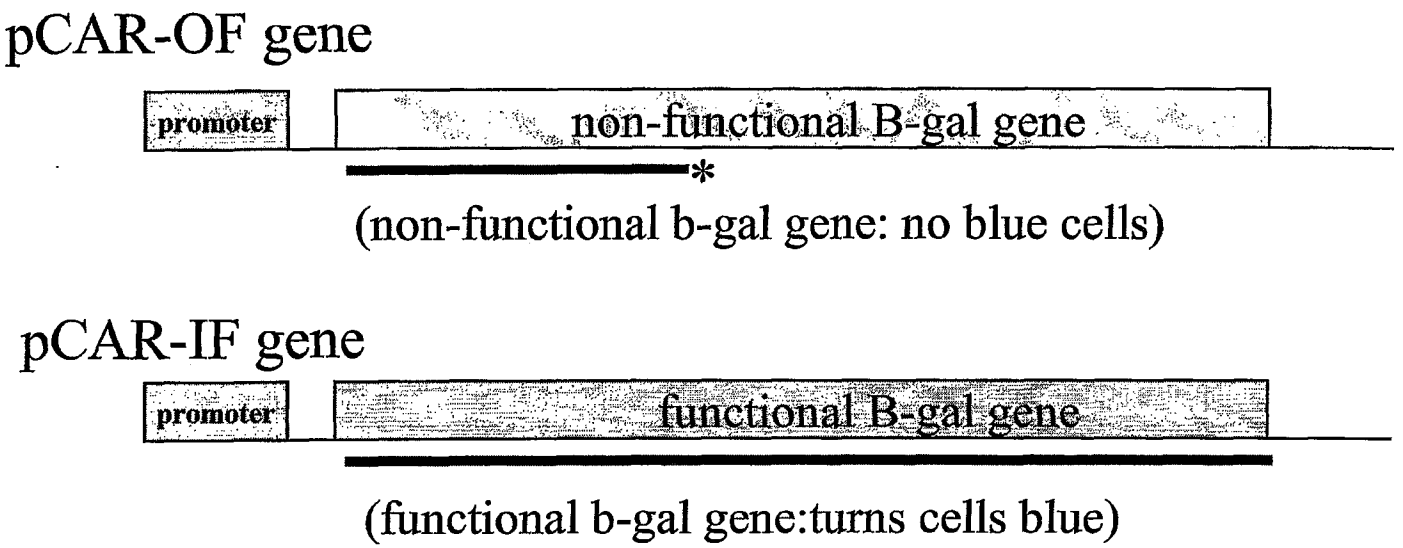


FIGURE 1. Engineered genes used to measure the *in vivo* gene altering capability of chemical induced defective mismatch repair. In MMR defective cells, the non-functional β-gal gene is altered to produce a functional protein that can turn cells blue in the presence of X-gal substrate.

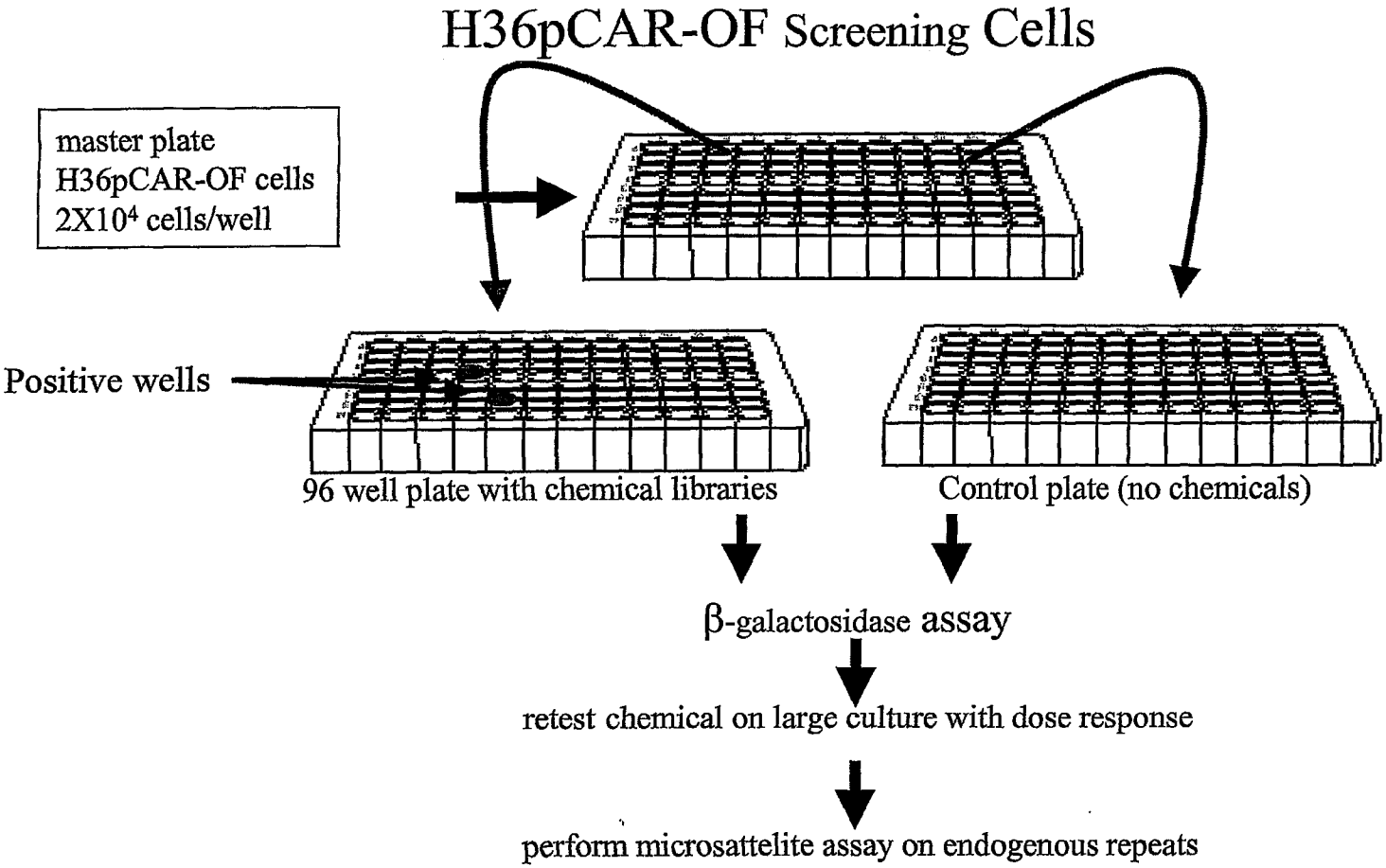


Figure 2: Screening method for identifying mismatch repair blocking chemicals. The assay employs the use of H36pCAR-OF cells which constitutively express the nonfunctional β-galactosidase pCAR-OF gene. Twenty thousand cells are plated in 100 µls of growth medium in a 96-well master plate 50µls of cells (ten thousand cells) are then replated into duplicate wells, one containing chemicals, the other control medium to account for background Cells are grown for 14 days, lysed and measured for β-galactosidase activity using CPRG substrate buffer Wells are measured for activity by spectrophometery at an OD of 576nm. Chemicals producing positive activity are then retested on larger H36pCAR-OF cultures at different doses. Cultures are measured for β-galactosidase and stability of endogenous microsattelite repeats.

Figure 3. DMA produces b-gal positive H36pCAR-OF cells. H36pCAR-OF cells Grown in the presence of DMA generated functional β-gal producing reporter Cells due to alteration of the polyA repeat contained within the N-terminus of the construct. The Arrow indicates β-gal positive cells. Approximately 3% of cells were positive for β-gal.

FIGURE 4. Shifting of endogenous microsatellites in human cells induced by DMA in human 293 cells. Cells were cultured in the presence of DMA for 14-17 days. Genomic DNA was isolated and BAT26 microsatellites were analyzed by PCR and gel electrophoresis. (A) Markers were analyzed by PCR using total genomic DNA from 40 samples of treated and untreated cells. Bottom band is the product with the expected wild type (WT) allele size. The asterisk indicates the presence of a new allele in cells treated with DMA. No new alleles were observed in control cells. (B) BAT26 markers from DMA-treated and untreated cells were amplified and cloned into T-tailed vectors. Recombinant clones were then reamplified using BAT26 primers and run on 4% agarose gels and stained with ethidium bromide. Shown is a representative sampling of clones whereby clones with altered molecular weights were observed in DMA treated cells (bottom panel) but not in control Cells (top panel). The asterisk indicates markers with altered molecular weight.

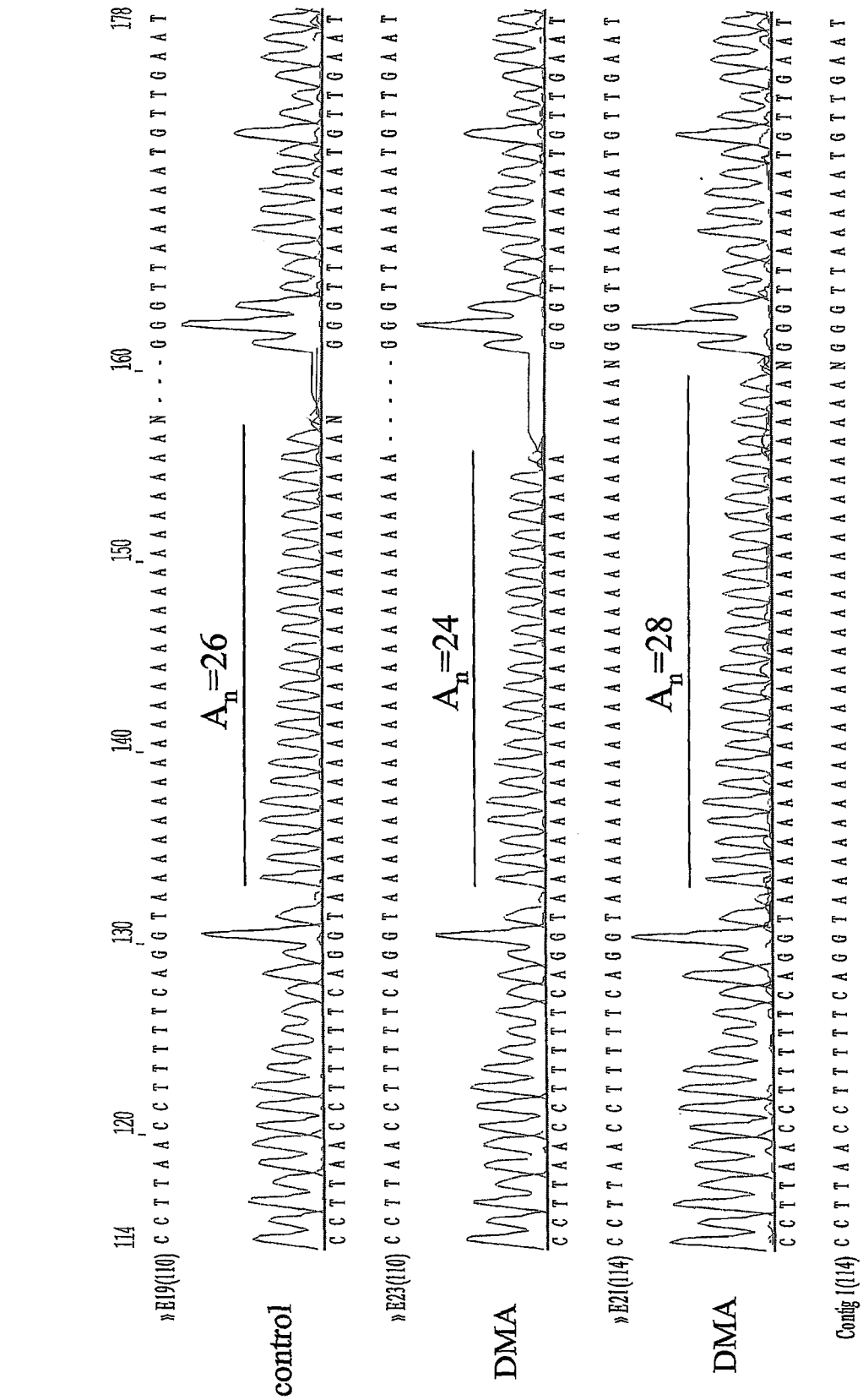


Figure 5. Sequence analysis of recombinant clones containing the BAT26 markers shows alterations within the endogenous polyA repeats in 293 cells treated with 250μm DMA but not in markers obtained from control cells (top sequence). Shown is a sequence alignment from 3 clones. Sequence was aligned using Vector NTI software.

Figure 6. Chemical inhibitors of MMR blocks spell check process leading to genetic alterations and new output traits. Shown here are offspring from control (WT) or DMA exposed *Arabidopsis thaliana* plants grown in standard soil conditions for 17 days. Six percent of the offspring from DMA treated plants had the small light green appearance. No plants with altered phenotypes were observed in the 150 plants from control or EMS mutagenized offspring. These data demonstrate the ability to generate a high rate of genetic alteration in host organisms by blockade of MMR *in vivo* that can lead to new output traits.

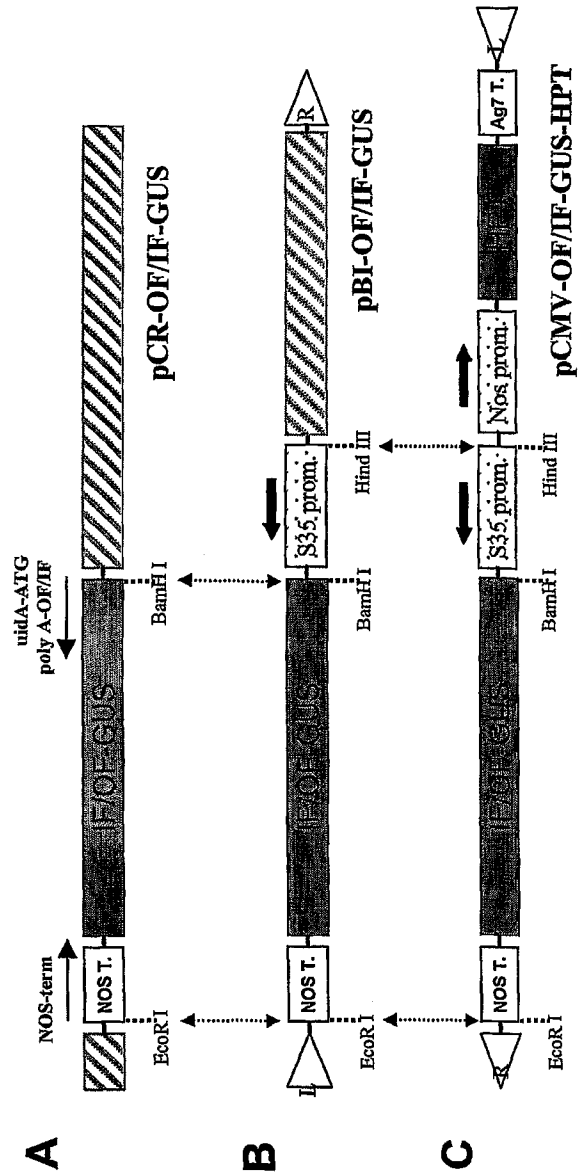


Figure 7. Binary vectors carrying the in-frame (IF) or out-of-frame (OF) version of the β-glucuronidase (GUS) gene. A) IF-GUS and OF-GUS genes, including the nopaline synthase terminator (NOS T.), were obtained by PCR using the NOS-term. and uidA-ATG poly A-OF/IF primers. PCR products were cloned in the TA cloning vector pCR2.1 and sequenced. B) IF-GUS or OF-GUS genes were then cloned into the EcoR I and BamH I sites of the pBI-121vector, which carries the Cauliflower Mosaic Virus S35 promoter (S35 prom.). C) The cassette containing the S35 promoter, the IF/OF-GUS gene, and the NOS T. was subsequently cloned into the EcoR I and Hind III sites of the pGPTV-HPT binary vector, to generate pCMV-IF-GUS-HPT or pCMV-OF-GUS-HPT constructs. HPT, hygromycin phosphotransferase gene. L, T-DNA left border. R, T-DNA right border. Solid arrows indicate direction of transcription. Dotted arrows indicate subcloning sites. Ag7, gene 7 terminator.

Figure 8. Examples of chemical inhibitors of mismatch repair. 9, 10 dimethyl anthracene and anthracene analogs are effective chemical inhibitors of mismatch repair *in vivo*.

SEQUENCE LISTING

<110> Nicolaides, Nicholas C
Grasso, Luigi
Sass, Philip M

<120> CHEMICAL INHIBITORS OF MISMATCH REPAIR

<130> MOR-0018

<140> 00/000,000
<141> 2001-01-15

<160> 44

<170> PatentIn Ver. 2.1

<210> 1
<211> 52
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 1
ggatcctaat acgactcact atagggagac caccatgtcg ttcgtggcag gg 52

<210> 2
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 2
taagtcttaa gtgctaccaa c 21

<210> 3
<211> 53
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 3
ggatcctaat acgactcact atagggagac caccatggaa caattgcctg cgg 53

<210> 4
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 4
aggttagtga agactctgtc 20

<210> 5
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 5
ctgatctcac ggacaatagt gc 22

<210> 6
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 6
ggctccataa aaagtgcacc 20

<210> 7

<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 7
ggtctgttga tgtcgtaagt cg 22

<210> 8
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 8
atcttgaaac ctttagggag gg 22

<210> 9
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 9
agaagtttag acaggtac 18

<210> 10
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 10

aaatgtgcaa ttgccttc 18

<210> 11
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 11
cccggatcca tgttaaaaaa aaaaaaaaaa aaaaaacgtc ctgtagaaac c 51

<210> 12
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 12
cccggatcca tgttaaaaaa aaaaaaaaaa aaaacgtcct gtagaaac 48

<210> 13
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 13
cccgaattcc ccgatctagt aacatagatg 30

<210> 14
<211> 859
<212> PRT
<213> Mus musculus

<400> 14

```
Met Glu Gln Thr Glu Gly Val Ser Thr Glu Cys Ala Lys Ala Ile Lys
 1               5                  10                  15

Pro Ile Asp Gly Lys Ser Val His Gln Ile Cys Ser Gly Gln Val Ile
            20                  25                  30

Leu Ser Leu Ser Thr Ala Val Lys Glu Leu Ile Glu Asn Ser Val Asp
        35                  40                  45

Ala Gly Ala Thr Thr Ile Asp Leu Arg Leu Lys Asp Tyr Gly Val Asp
    50                  55                  60

Leu Ile Glu Val Ser Asp Asn Gly Cys Gly Val Glu Glu Glu Asn Phe
65                  70                  75                  80

Glu Gly Leu Ala Leu Lys His His Thr Ser Lys Ile Gln Glu Phe Ala
                85                  90                  95

Asp Leu Thr Gln Val Glu Thr Phe Gly Phe Arg Gly Glu Ala Leu Ser
            100                 105                 110

Ser Leu Cys Ala Leu Ser Asp Val Thr Ile Ser Thr Cys His Gly Ser
        115                 120                 125

Ala Ser Val Gly Thr Arg Leu Val Phe Asp His Asn Gly Lys Ile Thr
    130                 135                 140

Gln Lys Thr Pro Tyr Pro Arg Pro Lys Gly Thr Thr Val Ser Val Gln
145                 150                 155                 160

His Leu Phe Tyr Thr Leu Pro Val Arg Tyr Lys Glu Phe Gln Arg Asn
                165                 170                 175

Ile Lys Lys Glu Tyr Ser Lys Met Val Gln Val Leu Gln Ala Tyr Cys
            180                 185                 190

Ile Ile Ser Ala Gly Val Arg Val Ser Cys Thr Asn Gln Leu Gly Gln
        195                 200                 205

Gly Lys Arg His Ala Val Val Cys Thr Ser Gly Thr Ser Gly Met Lys
    210                 215                 220

Glu Asn Ile Gly Ser Val Phe Gly Gln Lys Gln Leu Gln Ser Leu Ile
225                 230                 235                 240

Pro Phe Val Gln Leu Pro Pro Ser Asp Ala Val Cys Glu Glu Tyr Gly
                245                 250                 255
```

```
Leu Ser Thr Ser Gly Arg His Lys Thr Phe Ser Thr Phe Arg Ala Ser
            260                 265                 270

Phe His Ser Ala Arg Thr Ala Pro Gly Gly Val Gln Gln Thr Gly Ser
        275                 280                 285

Phe Ser Ser Ser Ile Arg Gly Pro Val Thr Gln Gln Arg Ser Leu Ser
    290                 295                 300

Leu Ser Met Arg Phe Tyr His Met Tyr Asn Arg His Gln Tyr Pro Phe
305                 310                 315                 320

Val Val Leu Asn Val Ser Val Asp Ser Glu Cys Val Asp Ile Asn Val
                325                 330                 335

Thr Pro Asp Lys Arg Gln Ile Leu Leu Gln Glu Glu Lys Leu Leu Leu
            340                 345                 350

Ala Val Leu Lys Thr Ser Leu Ile Gly Met Phe Asp Ser Asp Ala Asn
        355                 360                 365

Lys Leu Asn Val Asn Gln Gln Pro Leu Leu Asp Val Glu Gly Asn Leu
    370                 375                 380

Val Lys Leu His Thr Ala Glu Leu Glu Lys Pro Val Pro Gly Lys Gln
385                 390                 395                 400

Asp Asn Ser Pro Ser Leu Lys Ser Thr Ala Asp Glu Lys Arg Val Ala
                405                 410                 415

Ser Ile Ser Arg Leu Arg Glu Ala Phe Ser Leu His Pro Thr Lys Glu
            420                 425                 430

Ile Lys Ser Arg Gly Pro Glu Thr Ala Glu Leu Thr Arg Ser Phe Pro
        435                 440                 445

Ser Glu Lys Arg Gly Val Leu Ser Ser Tyr Pro Ser Asp Val Ile Ser
    450                 455                 460

Tyr Arg Gly Leu Arg Gly Ser Gln Asp Lys Leu Val Ser Pro Thr Asp
465                 470                 475                 480

Ser Pro Gly Asp Cys Met Asp Arg Glu Lys Ile Glu Lys Asp Ser Gly
                485                 490                 495

Leu Ser Ser Thr Ser Ala Gly Ser Glu Glu Glu Phe Ser Thr Pro Glu
            500                 505                 510
```

```
Val Ala Ser Ser Phe Ser Ser Asp Tyr Asn Val Ser Ser Leu Glu Asp
        515                 520                 525

Arg Pro Ser Gln Glu Thr Ile Asn Cys Gly Asp Leu Asp Cys Arg Pro
    530                 535                 540

Pro Gly Thr Gly Gln Ser Leu Lys Pro Glu Asp His Gly Tyr Gln Cys
545                 550                 555                 560

Lys Ala Leu Pro Leu Ala Arg Leu Ser Pro Thr Asn Ala Lys Arg Phe
                565                 570                 575

Lys Thr Glu Glu Arg Pro Ser Asn Val Asn Ile Ser Gln Arg Leu Pro
            580                 585                 590

Gly Pro Gln Ser Thr Ser Ala Ala Glu Val Asp Val Ala Ile Lys Met
        595                 600                 605

Asn Lys Arg Ile Val Leu Leu Glu Phe Ser Leu Ser Ser Leu Ala Lys
    610                 615                 620

Arg Met Lys Gln Leu Gln His Leu Lys Ala Gln Asn Lys His Glu Leu
625                 630                 635                 640

Ser Tyr Arg Lys Phe Arg Ala Lys Ile Cys Pro Gly Glu Asn Gln Ala
                645                 650                 655

Ala Glu Asp Glu Leu Arg Lys Glu Ile Ser Lys Ser Met Phe Ala Glu
            660                 665                 670

Met Glu Ile Leu Gly Gln Phe Asn Leu Gly Phe Ile Val Thr Lys Leu
        675                 680                 685

Lys Glu Asp Leu Phe Leu Val Asp Gln His Ala Ala Asp Glu Lys Tyr
    690                 695                 700

Asn Phe Glu Met Leu Gln Gln His Thr Val Leu Gln Ala Gln Arg Leu
705                 710                 715                 720

Ile Thr Pro Gln Thr Leu Asn Leu Thr Ala Val Asn Glu Ala Val Leu
                725                 730                 735

Ile Glu Asn Leu Glu Ile Phe Arg Lys Asn Gly Phe Asp Phe Val Ile
            740                 745                 750

Asp Glu Asp Ala Pro Val Thr Glu Arg Ala Lys Leu Ile Ser Leu Pro
        755                 760                 765
```

```
Thr Ser Lys Asn Trp Thr Phe Gly Pro Gln Asp Ile Asp Glu Leu Ile
    770                 775                 780

Phe Met Leu Ser Asp Ser Pro Gly Val Met Cys Arg Pro Ser Arg Val
785                 790                 795                 800

Arg Gln Met Phe Ala Ser Arg Ala Cys Arg Lys Ser Val Met Ile Gly
                805                 810                 815

Thr Ala Leu Asn Ala Ser Glu Met Lys Lys Leu Ile Thr His Met Gly
            820                 825                 830

Glu Met Asp His Pro Trp Asn Cys Pro His Gly Arg Pro Thr Met Arg
        835                 840                 845

His Val Ala Asn Leu Asp Val Ile Ser Gln Asn
    850                 855
```

<210> 15
<211> 3056
<212> DNA
<213> Mus musculus

<400> 15

```
gaattccggt gaaggtcctg aagaatttcc agattcctga gtatcattgg aggagacaga 60
taacctgtcg tcaggtaacg atggtgtata tgcaacagaa atgggtgttc ctggagacgc 120
gtcttttccc gagagcggca ccgcaactct cccgcggtga ctgtgactgg aggagtcctg 180
catccatgga gcaaaccgaa ggcgtgagta cagaatgtgc taaggccatc aagcctattg 240
atgggaagtc agtccatcaa atttgttctg ggcaggtgat actcagttta agcaccgctg 300
tgaaggagtt gatagaaaat agtgtagatg ctggtgctac tactattgat ctaaggctta 360
aagactatgg ggtggacctc attgaagttt cagacaatgg atgtggggta gaagaagaaa 420
actttgaagg tctagctctg aaacatcaca catctaagat tcaagagttt gccgacctca 480
cgcaggttga aactttcggc tttcgggggg aagctctgag ctctctgtgt gcactaagtg 540
atgtcactat atctacctgc cacgggtctg caagcgttgg gactcgactg gtgtttgacc 600
ataatgggaa aatcacccag aaaactccct acccccgacc taaaggaacc acagtcagtg 660
tgcagcactt attttataca ctacccgtgc gttacaaaga gtttcagagg aacattaaaa 720
aggagtattc caaaatggtg caggtcttac aggcgtactg tatcatctca gcaggcgtcc 780
gtgtaagctg cactaatcag ctcggacagg ggaagcggca cgctgtggtg tgcacaagcg 840
gcacgtctgg catgaaggaa aatatcgggt ctgtgtttgg ccagaagcag ttgcaaagcc 900
tcattccttt tgttcagctg ccccctagtg acgctgtgtg tgaagagtac ggcctgagca 960
cttcaggacg ccacaaaacc ttttctacgt ttcgggcttc atttcacagt gcacgcacgg 1020
cgccgggagg agtgcaacag acaggcagtt tttcttcatc aatcagaggc cctgtgaccc 1080
agcaaaggtc tctaagcttg tcaatgaggt tttatcacat gtataaccgg catcagtacc 1140
catttgtcgt ccttaacgtt tccgttgact cagaatgtgt ggatattaat gtaactccag 1200
ataaaaggca aattctacta caagaagaga agctattgct ggccgtttta aagacctcct 1260
tgataggaat gtttgacagt gatgcaaaca agcttaatgt caaccagcag ccactgctag 1320
atgttgaagg taacttagta aagctgcata ctgcagaact agaaaagcct gtgccaggaa 1380
```

agcaagataa ctctccttca ctgaagagca cagcagacga gaaaagggta gcatccatct 1440
ccaggctgag agaggccttt tctcttcatc ctactaaaga gatcaagtct aggggtccag 1500
agactgctga actgacacgg agttttccaa gtgagaaaag gggcgtgtta tcctcttatc 1560
cttcagacgt catctcttac agaggcctcc gtggctcgca ggacaaattg gtgagtccca 1620
cggacagccc tggtgactgt atggacagag agaaaataga aaaagactca gggctcagca 1680
gcacctcagc tggctctgag gaagagttca gcaccccaga agtggccagt agctttagca 1740
gtgactataa cgtgagctcc ctagaagaca gaccttctca ggaaaccata aactgtggtg 1800
acctggactg ccgtcctcca ggtacaggac agtccttgaa gccagaagac catggatatc 1860
aatgcaaagc tctacctcta gctcgtctgt cacccacaaa tgccaagcgc ttcaagacag 1920
aggaaagacc ctcaaatgtc aacatttctc aaagattgcc tggtcctcag agcacctcag 1980
cagctgaggt cgatgtagcc ataaaaatga ataagagaat cgtgctcctc gagttctctc 2040
tgagttctct agctaagcga atgaagcagt tacagcacct aaaggcgcag aacaaacatg 2100
aactgagtta cagaaaattt agggccaaga tttgccctgg agaaaaccaa gcagcagaag 2160
atgaactcag aaaagagatt agtaaatcga tgtttgcaga gatggagatc ttgggtcagt 2220
ttaacctggg atttatagta accaaactga aagaggacct cttcctggtg gaccagcatg 2280
ctgcggatga gaagtacaac tttgagatgc tgcagcagca cacggtgctc caggcgcaga 2340
ggctcatcac accccagact ctgaacttaa ctgctgtcaa tgaagctgta ctgatagaaa 2400
atctggaaat attcagaaag aatggctttg actttgtcat tgatgaggat gctccagtca 2460
ctgaaagggc taaattgatt tccttaccaa ctagtaaaaa ctggaccttt ggaccccaag 2520
atatagatga actgatcttt atgttaagtg acagccctgg ggtcatgtgc cggccctcac 2580
gagtcagaca gatgtttgct tccagagcct gtcggaagtc agtgatgatt ggaacggcgc 2640
tcaatgcgag cgagatgaag aagctcatca cccacatggg tgagatggac caccctgga 2700
actgccccca cggcaggcca accatgaggc acgttgccaa tctggatgtc atctctcaga 2760
actgacacac cccttgtagc atagagttta ttacagattg ttcggtttgc aaagagaagg 2820
ttttaagtaa tctgattatc gttgtacaaa aattagcatg ctgctttaat gtactggatc 2880
catttaaaag cagtgttaag gcaggcatga tggagtgttc ctctagctca gctacttggg 2940
tgatccggtg ggagctcatg tgagcccagg actttgagac cactccgagc cacattcatg 3000
agactcaatt caaggacaaa aaaaaaaaga tatttttgaa gccttttaaa aaaaaa 3056

<210> 16
<211> 862
<212> PRT
<213> Homo sapiens

<400> 16
Met Glu Arg Ala Glu Ser Ser Ser Thr Glu Pro Ala Lys Ala Ile Lys
1 5 10 15

Pro Ile Asp Arg Lys Ser Val His Gln Ile Cys Ser Gly Gln Val Val
20 25 30

Leu Ser Leu Ser Thr Ala Val Lys Glu Leu Val Glu Asn Ser Leu Asp
35 40 45

Ala Gly Ala Thr Asn Ile Asp Leu Lys Leu Lys Asp Tyr Gly Val Asp
50 55 60

```
Leu Ile Glu Val Ser Asp Asn Gly Cys Gly Val Glu Glu Glu Asn Phe
 65                  70                  75                  80

Glu Gly Leu Thr Leu Lys His His Thr Ser Lys Ile Gln Glu Phe Ala
                85                  90                  95

Asp Leu Thr Gln Val Glu Thr Phe Gly Phe Arg Gly Glu Ala Leu Ser
            100                 105                 110

Ser Leu Cys Ala Leu Ser Asp Val Thr Ile Ser Thr Cys His Ala Ser
        115                 120                 125

Ala Lys Val Gly Thr Arg Leu Met Phe Asp His Asn Gly Lys Ile Ile
    130                 135                 140

Gln Lys Thr Pro Tyr Pro Arg Pro Arg Gly Thr Thr Val Ser Val Gln
145                 150                 155                 160

Gln Leu Phe Ser Thr Leu Pro Val Arg His Lys Glu Phe Gln Arg Asn
                165                 170                 175

Ile Lys Lys Glu Tyr Ala Lys Met Val Gln Val Leu His Ala Tyr Cys
            180                 185                 190

Ile Ile Ser Ala Gly Ile Arg Val Ser Cys Thr Asn Gln Leu Gly Gln
        195                 200                 205

Gly Lys Arg Gln Pro Val Val Cys Thr Gly Gly Ser Pro Ser Ile Lys
    210                 215                 220

Glu Asn Ile Gly Ser Val Phe Gly Gln Lys Gln Leu Gln Ser Leu Ile
225                 230                 235                 240

Pro Phe Val Gln Leu Pro Pro Ser Asp Ser Val Cys Glu Glu Tyr Gly
                245                 250                 255

Leu Ser Cys Ser Asp Ala Leu His Asn Leu Phe Tyr Ile Ser Gly Phe
            260                 265                 270

Ile Ser Gln Cys Thr His Gly Val Gly Arg Ser Ser Thr Asp Arg Gln
        275                 280                 285

Phe Phe Phe Ile Asn Arg Arg Pro Cys Asp Pro Ala Lys Val Cys Arg
    290                 295                 300

Leu Val Asn Glu Val Tyr His Met Tyr Asn Arg His Gln Tyr Pro Phe
305                 310                 315                 320
```

Val Val Leu Asn Ile Ser Val Asp Ser Glu Cys Val Asp Ile Asn Val
325 330 335

Thr Pro Asp Lys Arg Gln Ile Leu Leu Gln Glu Glu Lys Leu Leu Leu
340 345 350

Ala Val Leu Lys Thr Ser Leu Ile Gly Met Phe Asp Ser Asp Val Asn
355 360 365

Lys Leu Asn Val Ser Gln Gln Pro Leu Leu Asp Val Glu Gly Asn Leu
370 375 380

Ile Lys Met His Ala Ala Asp Leu Glu Lys Pro Met Val Glu Lys Gln
385 390 395 400

Asp Gln Ser Pro Ser Leu Arg Thr Gly Glu Glu Lys Lys Asp Val Ser
405 410 415

Ile Ser Arg Leu Arg Glu Ala Phe Ser Leu Arg His Thr Thr Glu Asn
420 425 430

Lys Pro His Ser Pro Lys Thr Pro Glu Pro Arg Arg Ser Pro Leu Gly
435 440 445

Gln Lys Arg Gly Met Leu Ser Ser Ser Thr Ser Gly Ala Ile Ser Asp
450 455 460

Lys Gly Val Leu Arg Pro Gln Lys Glu Ala Val Ser Ser Ser His Gly
465 470 475 480

Pro Ser Asp Pro Thr Asp Arg Ala Glu Val Glu Lys Asp Ser Gly His
485 490 495

Gly Ser Thr Ser Val Asp Ser Glu Gly Phe Ser Ile Pro Asp Thr Gly
500 505 510

Ser His Cys Ser Ser Glu Tyr Ala Ala Ser Ser Pro Gly Asp Arg Gly
515 520 525

Ser Gln Glu His Val Asp Ser Gln Glu Lys Ala Pro Glu Thr Asp Asp
530 535 540

Ser Phe Ser Asp Val Asp Cys His Ser Asn Gln Glu Asp Thr Gly Cys
545 550 555 560

Lys Phe Arg Val Leu Pro Gln Pro Thr Asn Leu Ala Thr Pro Asn Thr
565 570 575

Lys Arg Phe Lys Lys Glu Glu Ile Leu Ser Ser Ser Asp Ile Cys Gln
580 585 590

Lys Leu Val Asn Thr Gln Asp Met Ser Ala Ser Gln Val Asp Val Ala
595 600 605

Val Lys Ile Asn Lys Lys Val Val Pro Leu Asp Phe Ser Met Ser Ser
610 615 620

Leu Ala Lys Arg Ile Lys Gln Leu His His Glu Ala Gln Gln Ser Glu
625 630 635 640

Gly Glu Gln Asn Tyr Arg Lys Phe Arg Ala Lys Ile Cys Pro Gly Glu
645 650 655

Asn Gln Ala Ala Glu Asp Glu Leu Arg Lys Glu Ile Ser Lys Thr Met
660 665 670

Phe Ala Glu Met Glu Ile Ile Gly Gln Phe Asn Leu Gly Phe Ile Ile
675 680 685

Thr Lys Leu Asn Glu Asp Ile Phe Ile Val Asp Gln His Ala Thr Asp
690 695 700

Glu Lys Tyr Asn Phe Glu Met Leu Gln Gln His Thr Val Leu Gln Gly
705 710 715 720

Gln Arg Leu Ile Ala Pro Gln Thr Leu Asn Leu Thr Ala Val Asn Glu
725 730 735

Ala Val Leu Ile Glu Asn Leu Glu Ile Phe Arg Lys Asn Gly Phe Asp
740 745 750

Phe Val Ile Asp Glu Asn Ala Pro Val Thr Glu Arg Ala Lys Leu Ile
755 760 765

Ser Leu Pro Thr Ser Lys Asn Trp Thr Phe Gly Pro Gln Asp Val Asp
770 775 780

Glu Leu Ile Phe Met Leu Ser Asp Ser Pro Gly Val Met Cys Arg Pro
785 790 795 800

Ser Arg Val Lys Gln Met Phe Ala Ser Arg Ala Cys Arg Lys Ser Val
805 810 815

Met Ile Gly Thr Ala Leu Asn Thr Ser Glu Met Lys Lys Leu Ile Thr
820 825 830

His Met Gly Glu Met Asp His Pro Trp Asn Cys Pro His Gly Arg Pro
835 840 845

Thr Met Arg His Ile Ala Asn Leu Gly Val Ile Ser Gln Asn
850 855 860

<210> 17
<211> 2771
<212> DNA
<213> Homo sapiens

<400> 17
cgaggcggat cgggtgttgc atccatggag cgagctgaga gctcgagtac agaacctgct 60
aaggccatca aacctattga tcggaagtca gtccatcaga tttgctctgg gcaggtggta 120
ctgagtctaa gcactgcggt aaaggagtta gtagaaaaca gtctggatgc tggtgccact 180
aatattgatc taaagcttaa ggactatgga gtggatctta ttgaagtttc agacaatgga 240
tgtggggtag aagaagaaaa cttcgaaggc ttaactctga aacatcacac atctaagatt 300
caagagtttg ccgacctaac tcaggttgaa acttttggct ttcgggggga agctctgagc 360
tcactttgtg cactgagcga tgtcaccatt tctacctgcc acgcatcggc gaaggttgga 420
actcgactga tgtttgatca caatgggaaa attatccaga aaacccccta ccccgccccc 480
agagggacca cagtcagcgt gcagcagtta ttttccacac tacctgtgcg ccataaggaa 540
tttcaaagga atattaagaa ggagtatgcc aaaatggtcc aggtcttaca tgcatactgt 600
atcatttcag caggcatccg tgtaagttgc accaatcagc ttggacaagg aaaacgacag 660
cctgtggtat gcacaggtgg aagccccagc ataaaggaaa atatcggctc tgtgtttggg 720
cagaagcagt tgcaaagcct cattcctttt gttcagctgc cccctagtga ctccgtgtgt 780
gaagagtacg gtttgagctg ttcggatgct ctgcataatc ttttttacat ctcaggtttc 840
atttcacaat gcacgcatgg agttggaagg agttcaacag acagacagtt tttctttatc 900
aaccggcggc cttgtgaccc agcaaaggtc tgcagactcg tgaatgaggt ctaccacatg 960
tataatcgac accagtatcc atttgttgtt cttaacattt ctgttgattc agaatgcgtt 1020
gatatcaatg ttactccaga taaaaggcaa attttgctac aagaggaaaa gcttttgttg 1080
gcagttttaa agacctcttt gataggaatg tttgatagtg atgtcaacaa gctaaatgtc 1140
agtcagcagc cactgctgga tgttgaaggt aacttaataa aaatgcatgc agcggatttg 1200
gaaaagccca tggtagaaaa gcaggatcaa tcccttcat taaggactgg agaagaaaaa 1260
aaagacgtgt ccatttccag actgcgagag gccttttctc ttcgtcacac aacagagaac 1320
aagcctcaca gcccaaagac tccagaacca agaaggagcc ctctaggaca gaaaaggggt 1380
atgctgtctt ctagcacttc aggtgccatc tctgacaaag gcgtcctgag acctcagaaa 1440
gaggcagtga gttccagtca cggacccagt gaccctacgg acagagcgga ggtggagaag 1500
gactcggggc acggcagcac ttccgtggat tctgaggggt tcagcatccc agacacgggc 1560
agtcactgca gcagcgagta tgcggccagc tccccagggg acaggggctc gcaggaacat 1620
gtggactctc aggagaaagc gcctgaaact gacgactctt tttcagatgt ggactgccat 1680
tcaaaccagg aagataccgg atgtaaattt cgagttttgc ctcagccaac taatctcgca 1740
accccaaaca caaagcgttt taaaaaagaa gaaattcttt ccagttctga catttgtcaa 1800
aagttagtaa atactcagga catgtcagcc tctcaggttg atgtagctgt gaaaattaat 1860
aagaaagttg tgcccctgga cttttctatg agttctttag ctaaacgaat aaagcagtta 1920
catcatgaag cacagcaaag tgaaggggaa cagaattaca ggaagtttag ggcaaagatt 1980
tgtcctggag aaaatcaagc agccgaagat gaactaagaa aagagataag taaaacgatg 2040
tttgcagaaa tggaaatcat tggtcagttt aacctgggat ttataataac caaactgaat 2100

gaggatatct tcatagtgga ccagcatgcc acggacgaga agtataactt cgagatgctg 2160
cagcagcaca ccgtgctcca ggggcagagg ctcatagcac ctcagactct caacttaact 2220
gctgttaatg aagctgttct gatagaaaat ctggaaatat ttagaaagaa tggctttgat 2280
tttgttatcg atgaaaatgc tccagtcact gaaagggcta aactgatttc cttgccaact 2340
agtaaaaact ggaccttcgg accccaggac gtcgatgaac tgatcttcat gctgagcgac 2400
agccctgggg tcatgtgccg gccttcccga gtcaagcaga tgtttgcctc cagagcctgc 2460
cggaagtcgg tgatgattgg gactgctctt aacacaagcg agatgaagaa actgatcacc 2520
cacatggggg agatggacca cccctggaac tgtccccatg gaaggccaac catgagacac 2580
atcgccaacc tgggtgtcat ttctcagaac tgaccgtagt cactgtatgg aataattggt 2640
tttatcgcag atttttatgt tttgaaagac agagtcttca ctaacctttt ttgttttaaa 2700
atgaaacctg ctacttaaaa aaaatacaca tcacacccat ttaaaagtga tcttgagaac 2760
cttttcaaac c 2771

<210> 18
<211> 932
<212> PRT
<213> Homo sapiens

<400> 18
Met Lys Gln Leu Pro Ala Ala Thr Val Arg Leu Leu Ser Ser Ser Gln
1 5 10 15

Ile Ile Thr Ser Val Val Ser Val Val Lys Glu Leu Ile Glu Asn Ser
20 25 30

Leu Asp Ala Gly Ala Thr Ser Val Asp Val Lys Leu Glu Asn Tyr Gly
35 40 45

Phe Asp Lys Ile Glu Val Arg Asp Asn Gly Glu Gly Ile Lys Ala Val
50 55 60

Asp Ala Pro Val Met Ala Met Lys Tyr Tyr Thr Ser Lys Ile Asn Ser
65 70 75 80

His Glu Asp Leu Glu Asn Leu Thr Thr Tyr Gly Phe Arg Gly Glu Ala
85 90 95

Leu Gly Ser Ile Cys Cys Ile Ala Glu Val Leu Ile Thr Thr Arg Thr
100 105 110

Ala Ala Asp Asn Phe Ser Thr Gln Tyr Val Leu Asp Gly Ser Gly His
115 120 125

Ile Leu Ser Gln Lys Pro Ser His Leu Gly Gln Gly Thr Thr Val Thr
130 135 140

Ala Leu Arg Leu Phe Lys Asn Leu Pro Val Arg Lys Gln Phe Tyr Ser

145 150 155 160

Thr Ala Lys Lys Cys Lys Asp Glu Ile Lys Lys Ile Gln Asp Leu Leu
165 170 175

Met Ser Phe Gly Ile Leu Lys Pro Asp Leu Arg Ile Val Phe Val His
180 185 190

Asn Lys Ala Val Ile Trp Gln Lys Ser Arg Val Ser Asp His Lys Met
195 200 205

Ala Leu Met Ser Val Leu Gly Thr Ala Val Met Asn Asn Met Glu Ser
210 215 220

Phe Gln Tyr His Ser Glu Glu Ser Gln Ile Tyr Leu Ser Gly Phe Leu
225 230 235 240

Pro Lys Cys Asp Ala Asp His Ser Phe Thr Ser Leu Ser Thr Pro Glu
245 250 255

Arg Ser Phe Ile Phe Ile Asn Ser Arg Pro Val His Gln Lys Asp Ile
260 265 270

Leu Lys Leu Ile Arg His His Tyr Asn Leu Lys Cys Leu Lys Glu Ser
275 280 285

Thr Arg Leu Tyr Pro Val Phe Phe Leu Lys Ile Asp Val Pro Thr Ala
290 295 300

Asp Val Asp Val Asn Leu Thr Pro Asp Lys Ser Gln Val Leu Leu Gln
305 310 315 320

Asn Lys Glu Ser Val Leu Ile Ala Leu Glu Asn Leu Met Thr Thr Cys
325 330 335

Tyr Gly Pro Leu Pro Ser Thr Asn Ser Tyr Glu Asn Asn Lys Thr Asp
340 345 350

Val Ser Ala Ala Asp Ile Val Leu Ser Lys Thr Ala Glu Thr Asp Val
355 360 365

Leu Phe Asn Lys Val Glu Ser Ser Gly Lys Asn Tyr Ser Asn Val Asp
370 375 380

Thr Ser Val Ile Pro Phe Gln Asn Asp Met His Asn Asp Glu Ser Gly
385 390 395 400

Lys Asn Thr Asp Asp Cys Leu Asn His Gln Ile Ser Ile Gly Asp Phe

405 410 415

Gly Tyr Gly His Cys Ser Ser Glu Ile Ser Asn Ile Asp Lys Asn Thr
420 425 430

Lys Asn Ala Phe Gln Asp Ile Ser Met Ser Asn Val Ser Trp Glu Asn
435 440 445

Ser Gln Thr Glu Tyr Ser Lys Thr Cys Phe Ile Ser Ser Val Lys His
450 455 460

Thr Gln Ser Glu Asn Gly Asn Lys Asp His Ile Asp Glu Ser Gly Glu
465 470 475 480

Asn Glu Glu Glu Ala Gly Leu Glu Asn Ser Ser Glu Ile Ser Ala Asp
485 490 495

Glu Trp Ser Arg Gly Asn Ile Leu Lys Asn Ser Val Gly Glu Asn Ile
500 505 510

Glu Pro Val Lys Ile Leu Val Pro Glu Lys Ser Leu Pro Cys Lys Val
515 520 525

Ser Asn Asn Asn Tyr Pro Ile Pro Glu Gln Met Asn Leu Asn Glu Asp
530 535 540

Ser Cys Asn Lys Lys Ser Asn Val Ile Asp Asn Lys Ser Gly Lys Val
545 550 555 560

Thr Ala Tyr Asp Leu Leu Ser Asn Arg Val Ile Lys Lys Pro Met Ser
565 570 575

Ala Ser Ala Leu Phe Val Gln Asp His Arg Pro Gln Phe Leu Ile Glu
580 585 590

Asn Pro Lys Thr Ser Leu Glu Asp Ala Thr Leu Gln Ile Glu Glu Leu
595 600 605

Trp Lys Thr Leu Ser Glu Glu Glu Lys Leu Lys Tyr Glu Glu Lys Ala
610 615 620

Thr Lys Asp Leu Glu Arg Tyr Asn Ser Gln Met Lys Arg Ala Ile Glu
625 630 635 640

Gln Glu Ser Gln Met Ser Leu Lys Asp Gly Arg Lys Lys Ile Lys Pro
645 650 655

Thr Ser Ala Trp Asn Leu Ala Gln Lys His Lys Leu Lys Thr Ser Leu

660 665 670

Ser Asn Gln Pro Lys Leu Asp Glu Leu Leu Gln Ser Gln Ile Glu Lys
675 680 685

Arg Arg Ser Gln Asn Ile Lys Met Val Gln Ile Pro Phe Ser Met Lys
690 695 700

Asn Leu Lys Ile Asn Phe Lys Lys Gln Asn Lys Val Asp Leu Glu Glu
705 710 715 720

Lys Asp Glu Pro Cys Leu Ile His Asn Leu Arg Phe Pro Asp Ala Trp
725 730 735

Leu Met Thr Ser Lys Thr Glu Val Met Leu Leu Asn Pro Tyr Arg Val
740 745 750

Glu Glu Ala Leu Leu Phe Lys Arg Leu Leu Glu Asn His Lys Leu Pro
755 760 765

Ala Glu Pro Leu Glu Lys Pro Ile Met Leu Thr Glu Ser Leu Phe Asn
770 775 780

Gly Ser His Tyr Leu Asp Val Leu Tyr Lys Met Thr Ala Asp Asp Gln
785 790 795 800

Arg Tyr Ser Gly Ser Thr Tyr Leu Ser Asp Pro Arg Leu Thr Ala Asn
805 810 815

Gly Phe Lys Ile Lys Leu Ile Pro Gly Val Ser Ile Thr Glu Asn Tyr
820 825 830

Leu Glu Ile Glu Gly Met Ala Asn Cys Leu Pro Phe Tyr Gly Val Ala
835 840 845

Asp Leu Lys Glu Ile Leu Asn Ala Ile Leu Asn Arg Asn Ala Lys Glu
850 855 860

Val Tyr Glu Cys Arg Pro Arg Lys Val Ile Ser Tyr Leu Glu Gly Glu
865 870 875 880

Ala Val Arg Leu Ser Arg Gln Leu Pro Met Tyr Leu Ser Lys Glu Asp
885 890 895

Ile Gln Asp Ile Ile Tyr Arg Met Lys His Gln Phe Gly Asn Glu Ile
900 905 910

Lys Glu Cys Val His Gly Arg Pro Phe Phe His His Leu Thr Tyr Leu

915 920 925

Pro Glu Thr Thr
930

<210> 19
<211> 3063
<212> DNA
<213> Homo sapiens

<400> 19
ggcacgagtg gctgcttgcg gctagtggat ggtaattgcc tgcctcgcgc tagcagcaag 60
ctgctctgtt aaaagcgaaa atgaaacaat tgcctgcggc aacagttcga ctcctttcaa 120
gttctcagat catcacttcg gtggtcagtg ttgtaaaaga gcttattgaa aactccttgg 180
atgctggtgc cacaagcgta gatgttaaac tggagaacta tggatttgat aaaattgagg 240
tgcgagataa cggggagggt atcaaggctg ttgatgcacc tgtaatggca atgaagtact 300
acacctcaaa aataaatagt catgaagatc ttgaaaattt gacaacttac ggttttcgtg 360
gagaagcctt ggggtcaatt tgttgtatag ctgaggtttt aattacaaca agaacggctg 420
ctgataattt tagcacccag tatgttttag atggcagtgg ccacatactt tctcagaaac 480
cttcacatct tggtcaaggt acaactgtaa ctgctttaag attatttaag aatctacctg 540
taagaaagca gttttactca actgcaaaaa aatgtaaaga tgaaataaaa aagatccaag 600
atctcctcat gagctttggt atccttaaac ctgacttaag gattgtcttt gtacataaca 660
aggcagttat ttggcagaaa agcagagtat cagatcacaa gatggctctc atgtcagttc 720
tggggactgc tgttatgaac aatatggaat cctttcagta ccactctgaa gaatctcaga 780
tttatctcag tggatttctt ccaaagtgtg atgcagacca ctctttcact agtctttcaa 840
caccagaaag aagtttcatc ttcataaaca gtcgaccagt acatcaaaaa gatatcttaa 900
agttaatccg acatcattac aatctgaaat gcctaaagga atctactcgt ttgtatcctg 960
ttttctttct gaaaatcgat gttcctacag ctgatgttga tgtaaattta acaccagata 1020
aaagccaagt attattacaa aataaggaat ctgttttaat tgctcttgaa aatctgatga 1080
cgacttgtta tggaccatta cctagtacaa attcttatga aaataataaa acagatgttt 1140
ccgcagctga catcgttctt agtaaaacag cagaaacaga tgtgcttttt aataaagtgg 1200
aatcatctgg aaagaattat tcaaatgttg atacttcagt cattccattc caaaatgata 1260
tgcataatga tgaatctgga aaaaacactg atgattgttt aaatcaccag ataagtattg 1320
gtgactttgg ttatggtcat tgtagtagtg aaatttctaa cattgataaa aacactaaga 1380
atgcatttca ggacatttca atgagtaatg tatcatggga gaactctcag acggaatata 1440
gtaaaacttg ttttataagt tccgttaagc acacccagtc agaaaatggc aataaagacc 1500
atatagatga gagtggggaa aatgaggaag aagcaggtct tgaaaactct tcggaaattt 1560
ctgcagatga gtggagcagg ggaaatatac ttaaaaattc agtgggagag aatattgaac 1620
ctgtgaaaat tttagtgcct gaaaaaagtt taccatgtaa agtaagtaat aataattatc 1680
caatccctga acaaatgaat cttaatgaag attcatgtaa caaaaaatca aatgtaatag 1740
ataataaatc tggaaaagtt acagcttatg atttacttag caatcgagta atcaagaaac 1800
ccatgtcagc aagtgctctt tttgttcaag atcatcgtcc tcagtttctc atagaaaatc 1860
ctaagactag tttagaggat gcaacactac aaattgaaga actgtggaag acattgagtg 1920
aagaggaaaa actgaaatat gaagagaagg ctactaaaga cttggaacga tacaatagtc 1980
aaatgaagag agccattgaa caggagtcac aaatgtcact aaaagatggc agaaaaaaga 2040
taaaacccac cagcgcatgg aatttggccc agaagcacaa gttaaaaacc tcattatcta 2100
atcaaccaaa acttgatgaa ctccttcagt cccaaattga aaaaagaagg agtcaaaata 2160

ttaaaatggt acagatcccc ttttctatga aaaacttaaa aataaatttt aagaaacaaa 2220
acaaagttga cttagaagag aaggatgaac cttgcttgat ccacaatctc aggtttcctg 2280
atgcatggct aatgacatcc aaaacagagg taatgttatt aaatccatat agagtagaag 2340
aagccctgct atttaaaaga cttcttgaga atcataaact tcctgcagag ccactggaaa 2400
agccaattat gttaacagag agtcttttta atggatctca ttatttagac gttttatata 2460
aaatgacagc agatgaccaa agatacagtg gatcaactta cctgtctgat cctcgtctta 2520
cagcgaatgg tttcaagata aaattgatac caggagtttc aattactgaa aattacttgg 2580
aaatagaagg aatggctaat tgtctcccat tctatggagt agcagattta aaagaaattc 2640
ttaatgctat attaaacaga aatgcaaagg aagtttatga atgtagacct cgcaaagtga 2700
taagttattt agagggagaa gcagtgcgtc tatccagaca attacccatg tacttatcaa 2760
aagaggacat ccaagacatt atctacagaa tgaagcacca gtttggaaat gaaattaaag 2820
agtgtgttca tggtcgccca ttttttcatc atttaaccta tcttccagaa actacatgat 2880
taaatatgtt taagaagatt agttaccatt gaaattggtt ctgtcataaa acagcatgag 2940
tctggtttta aattatcttt gtattatgtg tcacatggtt atttttaaa tgaggattca 3000
ctgacttgtt tttatattga aaaaagttcc acgtattgta gaaaacgtaa ataaactaat 3060
aac 3063

<210> 20
<211> 934
<212> PRT
<213> Homo sapiens

<400> 20
Met Ala Val Gln Pro Lys Glu Thr Leu Gln Leu Glu Ser Ala Ala Glu
1 5 10 15

Val Gly Phe Val Arg Phe Phe Gln Gly Met Pro Glu Lys Pro Thr Thr
20 25 30

Thr Val Arg Leu Phe Asp Arg Gly Asp Phe Tyr Thr Ala His Gly Glu
35 40 45

Asp Ala Leu Leu Ala Ala Arg Glu Val Phe Lys Thr Gln Gly Val Ile
50 55 60

Lys Tyr Met Gly Pro Ala Gly Ala Lys Asn Leu Gln Ser Val Val Leu
65 70 75 80

Ser Lys Met Asn Phe Glu Ser Phe Val Lys Asp Leu Leu Leu Val Arg
85 90 95

Gln Tyr Arg Val Glu Val Tyr Lys Asn Arg Ala Gly Asn Lys Ala Ser
100 105 110

Lys Glu Asn Asp Trp Tyr Leu Ala Tyr Lys Ala Ser Pro Gly Asn Leu
115 120 125

```
Ser Gln Phe Glu Asp Ile Leu Phe Gly Asn Asn Asp Met Ser Ala Ser
    130                 135                 140

Ile Gly Val Val Gly Val Lys Met Ser Ala Val Asp Gly Gln Arg Gln
145                 150                 155                 160

Val Gly Val Gly Tyr Val Asp Ser Ile Gln Arg Lys Leu Gly Leu Cys
                165                 170                 175

Glu Phe Pro Asp Asn Asp Gln Phe Ser Asn Leu Glu Ala Leu Leu Ile
            180                 185                 190

Gln Ile Gly Pro Lys Glu Cys Val Leu Pro Gly Gly Glu Thr Ala Gly
        195                 200                 205

Asp Met Gly Lys Leu Arg Gln Ile Ile Gln Arg Gly Gly Ile Leu Ile
    210                 215                 220

Thr Glu Arg Lys Lys Ala Asp Phe Ser Thr Lys Asp Ile Tyr Gln Asp
225                 230                 235                 240

Leu Asn Arg Leu Leu Lys Gly Lys Lys Gly Glu Gln Met Asn Ser Ala
                245                 250                 255

Val Leu Pro Glu Met Glu Asn Gln Val Ala Val Ser Ser Leu Ser Ala
            260                 265                 270

Val Ile Lys Phe Leu Glu Leu Leu Ser Asp Asp Ser Asn Phe Gly Gln
        275                 280                 285

Phe Glu Leu Thr Thr Phe Asp Phe Ser Gln Tyr Met Lys Leu Asp Ile
    290                 295                 300

Ala Ala Val Arg Ala Leu Asn Leu Phe Gln Gly Ser Val Glu Asp Thr
305                 310                 315                 320

Thr Gly Ser Gln Ser Leu Ala Ala Leu Leu Asn Lys Cys Lys Thr Pro
                325                 330                 335

Gln Gly Gln Arg Leu Val Asn Gln Trp Ile Lys Gln Pro Leu Met Asp
            340                 345                 350

Lys Asn Arg Ile Glu Glu Arg Leu Asn Leu Val Glu Ala Phe Val Glu
        355                 360                 365

Asp Ala Glu Leu Arg Gln Thr Leu Gln Glu Asp Leu Leu Arg Arg Phe
    370                 375                 380
```

```
Pro Asp Leu Asn Arg Leu Ala Lys Lys Phe Gln Arg Gln Ala Ala Asn
385                 390                 395                 400

Leu Gln Asp Cys Tyr Arg Leu Tyr Gln Gly Ile Asn Gln Leu Pro Asn
                405                 410                 415

Val Ile Gln Ala Leu Glu Lys His Glu Gly Lys His Gln Lys Leu Leu
            420                 425                 430

Leu Ala Val Phe Val Thr Pro Leu Thr Asp Leu Arg Ser Asp Phe Ser
        435                 440                 445

Lys Phe Gln Glu Met Ile Glu Thr Thr Leu Asp Met Asp Gln Val Glu
    450                 455                 460

Asn His Glu Phe Leu Val Lys Pro Ser Phe Asp Pro Asn Leu Ser Glu
465                 470                 475                 480

Leu Arg Glu Ile Met Asn Asp Leu Glu Lys Lys Met Gln Ser Thr Leu
                485                 490                 495

Ile Ser Ala Ala Arg Asp Leu Gly Leu Asp Pro Gly Lys Gln Ile Lys
            500                 505                 510

Leu Asp Ser Ser Ala Gln Phe Gly Tyr Tyr Phe Arg Val Thr Cys Lys
        515                 520                 525

Glu Glu Lys Val Leu Arg Asn Asn Lys Asn Phe Ser Thr Val Asp Ile
    530                 535                 540

Gln Lys Asn Gly Val Lys Phe Thr Asn Ser Lys Leu Thr Ser Leu Asn
545                 550                 555                 560

Glu Glu Tyr Thr Lys Asn Lys Thr Glu Tyr Glu Glu Ala Gln Asp Ala
                565                 570                 575

Ile Val Lys Glu Ile Val Asn Ile Ser Ser Gly Tyr Val Glu Pro Met
            580                 585                 590

Gln Thr Leu Asn Asp Val Leu Ala Gln Leu Asp Ala Val Val Ser Phe
        595                 600                 605

Ala His Val Ser Asn Gly Ala Pro Val Pro Tyr Val Arg Pro Ala Ile
    610                 615                 620

Leu Glu Lys Gly Gln Gly Arg Ile Ile Leu Lys Ala Ser Arg His Ala
625                 630                 635                 640
```

Cys Val Glu Val Gln Asp Glu Ile Ala Phe Ile Pro Asn Asp Val Tyr
645 650 655

Phe Glu Lys Asp Lys Gln Met Phe His Ile Ile Thr Gly Pro Asn Met
660 665 670

Gly Gly Lys Ser Thr Tyr Ile Arg Gln Thr Gly Val Ile Val Leu Met
675 680 685

Ala Gln Ile Gly Cys Phe Val Pro Cys Glu Ser Ala Glu Val Ser Ile
690 695 700

Val Asp Cys Ile Leu Ala Arg Val Gly Ala Gly Asp Ser Gln Leu Lys
705 710 715 720

Gly Val Ser Thr Phe Met Ala Glu Met Leu Glu Thr Ala Ser Ile Leu
725 730 735

Arg Ser Ala Thr Lys Asp Ser Leu Ile Ile Ile Asp Glu Leu Gly Arg
740 745 750

Gly Thr Ser Thr Tyr Asp Gly Phe Gly Leu Ala Trp Ala Ile Ser Glu
755 760 765

Tyr Ile Ala Thr Lys Ile Gly Ala Phe Cys Met Phe Ala Thr His Phe
770 775 780

His Glu Leu Thr Ala Leu Ala Asn Gln Ile Pro Thr Val Asn Asn Leu
785 790 795 800

His Val Thr Ala Leu Thr Thr Glu Glu Thr Leu Thr Met Leu Tyr Gln
805 810 815

Val Lys Lys Gly Val Cys Asp Gln Ser Phe Gly Ile His Val Ala Glu
820 825 830

Leu Ala Asn Phe Pro Lys His Val Ile Glu Cys Ala Lys Gln Lys Ala
835 840 845

Leu Glu Leu Glu Glu Phe Gln Tyr Ile Gly Glu Ser Gln Gly Tyr Asp
850 855 860

Ile Met Glu Pro Ala Ala Lys Lys Cys Tyr Leu Glu Arg Glu Gln Gly
865 870 875 880

Glu Lys Ile Ile Gln Glu Phe Leu Ser Lys Val Lys Gln Met Pro Phe
885 890 895

Thr Glu Met Ser Glu Glu Asn Ile Thr Ile Lys Leu Lys Gln Leu Lys
900 905 910

Ala Glu Val Ile Ala Lys Asn Asn Ser Phe Val Asn Glu Ile Ile Ser
915 920 925

Arg Ile Lys Val Thr Thr
930

<210> 21
<211> 3145
<212> DNA
<213> Homo sapiens

<400> 21
ggcgggaaac agcttagtgg gtgtggggtc gcgcattttc ttcaaccagg aggtgaggag 60
gtttcgacat ggcggtgcag ccgaaggaga cgctgcagtt ggagagcgcg gccgaggtcg 120
gcttcgtgcg cttctttcag ggcatgccgg agaagccgac caccacagtg cgccttttcg 180
accggggcga cttctatacg gcgcacggcg aggacgcgct gctggccgcc cgggaggtgt 240
tcaagaccca gggggtgatc aagtacatgg ggccggcagg agcaaagaat ctgcagagtg 300
ttgtgcttag taaaatgaat tttgaatctt ttgtaaaaga tcttcttctg gttcgtcagt 360
atagagttga agtttataag aatagagctg gaaataaggc atccaaggag aatgattggt 420
atttggcata taaggcttct cctggcaatc tctctcagtt tgaagacatt ctctttggta 480
acaatgatat gtcagcttcc attggtgttg tgggtgttaa aatgtccgca gttgatggcc 540
agagacaggt tggagttggg tatgtggatt ccatacagag gaaactagga ctgtgtgaat 600
tccctgataa tgatcagttc tccaatcttg aggctctcct catccagatt ggaccaaagg 660
aatgtgtttt acccggagga gagactgctg gagacatggg gaaactgaga cagataattc 720
aaagaggagg aattctgatc acagaaagaa aaaaagctga ctttttccaca aaagacattt 780
atcaggacct caaccggttg ttgaaaggca aaaagggaga gcagatgaat agtgctgtat 840
tgccagaaat ggagaatcag gttgcagttt catcactgtc tgcggtaatc aagtttttag 900
aactcttatc agatgattcc aactttggac agtttgaact gactactttt gacttcagcc 960
agtatatgaa attggatatt gcagcagtca gagcccttaa ccttttttcag ggttctgttg 1020
aagataccac tggctctcag tctctggctg ccttgctgaa taagtgtaaa acccctcaag 1080
gacaaagact tgttaaccag tggattaagc agcctctcat ggataagaac agaatagagg 1140
agagattgaa tttagtggaa gcttttgtag aagatgcaga attgaggcag actttacaag 1200
aagatttact tcgtcgattc ccagatctta accgacttgc caagaagttt caaagacaag 1260
cagcaaactt acaagattgt taccgactct atcagggtat aaatcaacta cctaatgtta 1320
tacaggctct ggaaaaacat gaaggaaaac accagaaatt attgttggca gtttttgtga 1380
ctcctcttac tgatcttcgt tctgacttct ccaagtttca ggaaatgata gaaacaactt 1440
tagatatgga tcaggtggaa aaccatgaat tccttgtaaa accttcattt gatcctaatc 1500
tcagtgaatt aagagaaata atgaatgact tggaaaagaa gatgcagtca acattaataa 1560
gtgcagccag agatcttggc ttggaccctg gcaaacagat taaactggat tccagtgcac 1620
agtttggata ttactttcgt gtaacctgta aggaagaaaa agtccttcgt aacaataaaa 1680
actttagtac tgtagatatc cagaagaatg gtgttaaatt taccaacagc aaattgactt 1740
ctttaaatga agagtatacc aaaaataaaa cagaatatga agaagcccag gatgccattg 1800
ttaaagaaat tgtcaatatt tcttcaggct atgtagaacc aatgcagaca ctcaatgatg 1860
tgttagctca gctagatgct gttgtcagct ttgctcacgt gtcaaatgga gcacctgttc 1920

catatgtacg accagccatt ttggagaaag gacaaggaag aattatatta aaagcatcca 1980
ggcatgcttg tgttgaagtt caagatgaaa ttgcatttat tcctaatgac gtatactttg 2040
aaaaagataa acagatgttc cacatcatta ctggccccaa tatgggaggt aaatcaacat 2100
atattcgaca aactggggtg atagtactca tggcccaaat tgggtgtttt gtgccatgtg 2160
agtcagcaga agtgtccatt gtggactgca tcttagcccg agtaggggct ggtgacagtc 2220
aattgaaagg agtctccacg ttcatggctg aaatgttgga aactgcttct atcctcaggt 2280
ctgcaaccaa agattcatta ataatcatag atgaattggg aagaggaact tctacctacg 2340
atggatttgg gttagcatgg gctatatcag aatacattgc aacaaagatt ggtgcttttt 2400
gcatgtttgc aacccatttt catgaactta ctgccttggc caatcagata ccaactgtta 2460
ataatctaca tgtcacagca ctcaccactg aagagacctt aactatgctt tatcaggtga 2520
agaaaggtgt ctgtgatcaa agttttggga ttcatgttgc agagcttgct aatttcccta 2580
agcatgtaat agagtgtgct aaacagaaag ccctggaact tgaggagttt cagtatattg 2640
gagaatcgca aggatatgat atcatggaac cagcagcaaa gaagtgctat ctggaaagag 2700
agcaaggtga aaaaattatt caggagttcc tgtccaaggt gaaacaaatg ccctttactg 2760
aaatgtcaga agaaaacatc acaataaagt taaaacagct aaaagctgaa gtaatagcaa 2820
agaataatag ctttgtaaat gaaatcattt cacgaataaa agttactacg tgaaaaatcc 2880
cagtaatgga atgaaggtaa tattgataag ctattgtctg taatagtttt atattgtttt 2940
atattaaccc ttttttccata gtgttaactg tcagtgccca tgggctatca acttaataag 3000
atatttagta atattttact ttgaggacat tttcaaagat ttttattttg aaaaatgaga 3060
gctgtaactg aggactgttt gcaattgaca taggcaataa taagtgatgt gctgaatttt 3120
ataaataaaa tcatgtagtt tgtgg 3145

<210> 22
<211> 756
<212> PRT
<213> Homo sapiens

<400> 22
Met Ser Phe Val Ala Gly Val Ile Arg Arg Leu Asp Glu Thr Val Val
1 5 10 15

Asn Arg Ile Ala Ala Gly Glu Val Ile Gln Arg Pro Ala Asn Ala Ile
20 25 30

Lys Glu Met Ile Glu Asn Cys Leu Asp Ala Lys Ser Thr Ser Ile Gln
35 40 45

Val Ile Val Lys Glu Gly Gly Leu Lys Leu Ile Gln Ile Gln Asp Asn
50 55 60

Gly Thr Gly Ile Arg Lys Glu Asp Leu Asp Ile Val Cys Glu Arg Phe
65 70 75 80

Thr Thr Ser Lys Leu Gln Ser Phe Glu Asp Leu Ala Ser Ile Ser Thr
85 90 95

Tyr Gly Phe Arg Gly Glu Ala Leu Ala Ser Ile Ser His Val Ala His

```
            100                 105                 110

Val Thr Ile Thr Thr Lys Thr Ala Asp Gly Lys Cys Ala Tyr Arg Ala
        115                 120                 125

Ser Tyr Ser Asp Gly Lys Leu Lys Ala Pro Pro Lys Pro Cys Ala Gly
    130                 135                 140

Asn Gln Gly Thr Gln Ile Thr Val Glu Asp Leu Phe Tyr Asn Ile Ala
145                 150                 155                 160

Thr Arg Arg Lys Ala Leu Lys Asn Pro Ser Glu Glu Tyr Gly Lys Ile
                165                 170                 175

Leu Glu Val Val Gly Arg Tyr Ser Val His Asn Ala Gly Ile Ser Phe
            180                 185                 190

Ser Val Lys Lys Gln Gly Glu Thr Val Ala Asp Val Arg Thr Leu Pro
        195                 200                 205

Asn Ala Ser Thr Val Asp Asn Ile Arg Ser Ile Phe Gly Asn Ala Val
    210                 215                 220

Ser Arg Glu Leu Ile Glu Ile Gly Cys Glu Asp Lys Thr Leu Ala Phe
225                 230                 235                 240

Lys Met Asn Gly Tyr Ile Ser Asn Ala Asn Tyr Ser Val Lys Lys Cys
                245                 250                 255

Ile Phe Leu Leu Phe Ile Asn His Arg Leu Val Glu Ser Thr Ser Leu
            260                 265                 270

Arg Lys Ala Ile Glu Thr Val Tyr Ala Ala Tyr Leu Pro Lys Asn Thr
        275                 280                 285

His Pro Phe Leu Tyr Leu Ser Leu Glu Ile Ser Pro Gln Asn Val Asp
    290                 295                 300

Val Asn Val His Pro Thr Lys His Glu Val His Phe Leu His Glu Glu
305                 310                 315                 320

Ser Ile Leu Glu Arg Val Gln Gln His Ile Glu Ser Lys Leu Leu Gly
                325                 330                 335

Ser Asn Ser Ser Arg Met Tyr Phe Thr Gln Thr Leu Leu Pro Gly Leu
            340                 345                 350

Ala Gly Pro Ser Gly Glu Met Val Lys Ser Thr Thr Ser Leu Thr Ser
```

```
        355                 360                 365

Ser Ser Thr Ser Gly Ser Ser Asp Lys Val Tyr Ala His Gln Met Val
    370                 375                 380

Arg Thr Asp Ser Arg Glu Gln Lys Leu Asp Ala Phe Leu Gln Pro Leu
385                 390                 395                 400

Ser Lys Pro Leu Ser Ser Gln Pro Gln Ala Ile Val Thr Glu Asp Lys
                405                 410                 415

Thr Asp Ile Ser Ser Gly Arg Ala Arg Gln Gln Asp Glu Glu Met Leu
            420                 425                 430

Glu Leu Pro Ala Pro Ala Glu Val Ala Ala Lys Asn Gln Ser Leu Glu
        435                 440                 445

Gly Asp Thr Thr Lys Gly Thr Ser Glu Met Ser Glu Lys Arg Gly Pro
    450                 455                 460

Thr Ser Ser Asn Pro Arg Lys Arg His Arg Glu Asp Ser Asp Val Glu
465                 470                 475                 480

Met Val Glu Asp Asp Ser Arg Lys Glu Met Thr Ala Ala Cys Thr Pro
                485                 490                 495

Arg Arg Arg Ile Ile Asn Leu Thr Ser Val Leu Ser Leu Gln Glu Glu
            500                 505                 510

Ile Asn Glu Gln Gly His Glu Val Leu Arg Glu Met Leu His Asn His
        515                 520                 525

Ser Phe Val Gly Cys Val Asn Pro Gln Trp Ala Leu Ala Gln His Gln
    530                 535                 540

Thr Lys Leu Tyr Leu Leu Asn Thr Thr Lys Leu Ser Glu Glu Leu Phe
545                 550                 555                 560

Tyr Gln Ile Leu Ile Tyr Asp Phe Ala Asn Phe Gly Val Leu Arg Leu
                565                 570                 575

Ser Glu Pro Ala Pro Leu Phe Asp Leu Ala Met Leu Ala Leu Asp Ser
            580                 585                 590

Pro Glu Ser Gly Trp Thr Glu Glu Asp Gly Pro Lys Glu Gly Leu Ala
        595                 600                 605

Glu Tyr Ile Val Glu Phe Leu Lys Lys Lys Ala Glu Met Leu Ala Asp
```

610 615 620

Tyr Phe Ser Leu Glu Ile Asp Glu Glu Gly Asn Leu Ile Gly Leu Pro
625 630 635 640

Leu Leu Ile Asp Asn Tyr Val Pro Pro Leu Glu Gly Leu Pro Ile Phe
645 650 655

Ile Leu Arg Leu Ala Thr Glu Val Asn Trp Asp Glu Glu Lys Glu Cys
660 665 670

Phe Glu Ser Leu Ser Lys Glu Cys Ala Met Phe Tyr Ser Ile Arg Lys
675 680 685

Gln Tyr Ile Ser Glu Glu Ser Thr Leu Ser Gly Gln Gln Ser Glu Val
690 695 700

Pro Gly Ser Ile Pro Asn Ser Trp Lys Trp Thr Val Glu His Ile Val
705 710 715 720

Tyr Lys Ala Leu Arg Ser His Ile Leu Pro Pro Lys His Phe Thr Glu
725 730 735

Asp Gly Asn Ile Leu Gln Leu Ala Asn Leu Pro Asp Leu Tyr Lys Val
740 745 750

Phe Glu Arg Cys
755

<210> 23
<211> 2484
<212> DNA
<213> Homo sapiens

<400> 23
cttggctctt ctggcgccaa aatgtcgttc gtggcagggg ttattcggcg gctggacgag 60
acagtggtga accgcatcgc ggcgggggaa gttatccagc ggccagctaa tgctatcaaa 120
gagatgattg agaactgttt agatgcaaaa tccacaagta ttcaagtgat tgttaaagag 180
ggaggcctga agttgattca gatccaagac aatggcaccg ggatcaggaa agaagatctg 240
gatattgtat gtgaaaggtt cactactagt aaactgcagt cctttgagga tttagccagt 300
atttctacct atggctttcg aggtgaggct ttggccagca taagccatgt ggctcatgtt 360
actattacaa cgaaaacagc tgatggaaag tgtgcataca gagcaagtta ctcagatgga 420
aaactgaaag cccctcctaa accatgtgct ggcaatcaag ggacccagat cacggtggag 480
gacctttttt acaacatagc cacgaggaga aaagctttaa aaaatccaag tgaagaatat 540
gggaaaattt tggaagttgt tggcaggtat tcagtacaca atgcaggcat tagtttctca 600
gttaaaaaac aaggagagac agtagctgat gttaggacac tacccaatgc ctcaaccgtg 660
gacaatattc gctccatctt tggaaatgct gttagtcgag aactgataga aattggatgt 720

gaggataaaa ccctagcctt caaaatgaat ggttacatat ccaatgcaaa ctactcagtg 780
aagaagtgca tcttcttact cttcatcaac catcgtctgg tagaatcaac ttccttgaga 840
aaagccatag aaacagtgta tgcagcctat ttgcccaaaa acacacaccc attcctgtac 900
ctcagtttag aaatcagtcc ccagaatgtg gatgttaatg tgcaccccac aaagcatgaa 960
gttcacttcc tgcacgagga gagcatcctg gagcgggtgc agcagcacat cgagagcaag 1020
ctcctgggct ccaattcctc caggatgtac ttcacccaga ctttgctacc aggacttgct 1080
ggcccctctg gggagatggt taaatccaca acaagtctga cctcgtcttc tacttctgga 1140
agtagtgata aggtctatgc ccaccagatg gttcgtacag attcccggga acagaagctt 1200
gatgcatttc tgcagcctct gagcaaaccc ctgtccagtc agccccaggc cattgtcaca 1260
gaggataaga cagatatttc tagtggcagg gctaggcagc aagatgagga gatgcttgaa 1320
ctcccagccc ctgctgaagt ggctgccaaa aatcagagct tggaggggga tacaacaaag 1380
gggacttcag aaatgtcaga gaagagagga cctacttcca gcaaccccag aaagagacat 1440
cgggaagatt ctgatgtgga aatggtggaa gatgattccc gaaaggaaat gactgcagct 1500
tgtacccccc ggagaaggat cattaacctc actagtgttt tgagtctcca ggaagaaatt 1560
aatgagcagg gacatgaggt tctccgggag atgttgcata accactcctt cgtgggctgt 1620
gtgaatcctc agtgggcctt ggcacagcat caaaccaagt tataccttct caacaccacc 1680
aagcttagtg aagaactgtt ctaccagata ctcatttatg attttgccaa ttttggtgtt 1740
ctcaggttat cggagccagc accgctcttt gaccttgcca tgcttgcctt agatagtcca 1800
gagagtggct ggacagagga agatggtccc aaagaaggac ttgctgaata cattgttgag 1860
tttctgaaga agaaggctga gatgcttgca gactatttct ctttggaaat tgatgaggaa 1920
gggaacctga ttggattacc ccttctgatt gacaactatg tgccccctt ggagggactg 1980
cctatcttca ttcttcgact agccactgag gtgaattggg acgaagaaaa ggaatgtttt 2040
gaaagcctca gtaaagaatg cgctatgttc tattccatcc ggaagcagta catatctgag 2100
gagtcgaccc tctcaggcca gcagagtgaa gtgcctggct ccattccaaa ctcctggaag 2160
tggactgtgg aacacattgt ctataaagcc ttgcgctcac acattctgcc tcctaaacat 2220
ttcacagaag atggaaatat cctgcagctt gctaacctgc ctgatctata caaagtcttt 2280
gagaggtgtt aaatatggtt atttatgcac tgtgggatgt gttcttcttt ctctgtattc 2340
cgatacaaag tgttgtatca aagtgtgata tacaaagtgt accaacataa gtgttggtag 2400
cacttaagac ttatacttgc cttctgatag tattccttta tacacagtgg attgattata 2460
aataaataga tgtgtcttaa cata 2484

<210> 24
<211> 133
<212> PRT
<213> Homo sapiens

<400> 24
Met Lys Gln Leu Pro Ala Ala Thr Val Arg Leu Leu Ser Ser Ser Gln
1 5 10 15

Ile Ile Thr Ser Val Val Ser Val Val Lys Glu Leu Ile Glu Asn Ser
20 25 30

Leu Asp Ala Gly Ala Thr Ser Val Asp Val Lys Leu Glu Asn Tyr Gly
35 40 45

Phe Asp Lys Ile Glu Val Arg Asp Asn Gly Glu Gly Ile Lys Ala Val

50 55 60

Asp Ala Pro Val Met Ala Met Lys Tyr Tyr Thr Ser Lys Ile Asn Ser
65 70 75 80

His Glu Asp Leu Glu Asn Leu Thr Thr Tyr Gly Phe Arg Gly Glu Ala
85 90 95

Leu Gly Ser Ile Cys Cys Ile Ala Glu Val Leu Ile Thr Thr Arg Thr
100 105 110

Ala Ala Asp Asn Phe Ser Thr Gln Tyr Val Leu Asp Gly Ser Gly His
115 120 125

Ile Leu Ser Gln Lys
130

<210> 25
<211> 426
<212> DNA
<213> Homo sapiens

<400> 25
cgaggcggat cgggtgttgc atccatggag cgagctgaga gctcgagtac agaacctgct 60
aaggccatca aacctattga tcggaagtca gtccatcaga tttgctctgg gcaggtggta 120
ctgagtctaa gcactgcggt aaaggagtta gtagaaaaca gtctggatgc tggtgccact 180
aatattgatc taaagcttaa ggactatgga gtggatctta ttgaagtttc agacaatgga 240
tgtggggtag aagaagaaaa cttcgaaggc ttaactctga aacatcacac atctaagatt 300
caagagtttg ccgacctaac tcaggttgaa acttttggct ttcgggggga agctctgagc 360
tcactttgtg cactgagcga tgtcaccatt tctacctgcc acgcatcggc gaaggttgga 420
acttga 426

<210> 26
<211> 1360
<212> PRT
<213> Homo sapiens

<400> 26
Met Ser Arg Gln Ser Thr Leu Tyr Ser Phe Phe Pro Lys Ser Pro Ala
1 5 10 15

Leu Ser Asp Ala Asn Lys Ala Ser Ala Arg Ala Ser Arg Glu Gly Gly
20 25 30

Arg Ala Ala Ala Ala Pro Gly Ala Ser Pro Ser Pro Gly Gly Asp Ala
35 40 45

```
Ala Trp Ser Glu Ala Gly Pro Gly Pro Arg Pro Leu Ala Arg Ser Ala
     50                  55                  60

Ser Pro Pro Lys Ala Lys Asn Leu Asn Gly Gly Leu Arg Arg Ser Val
 65                  70                  75                  80

Ala Pro Ala Ala Pro Thr Ser Cys Asp Phe Ser Pro Gly Asp Leu Val
                85                  90                  95

Trp Ala Lys Met Glu Gly Tyr Pro Trp Trp Pro Cys Leu Val Tyr Asn
            100                 105                 110

His Pro Phe Asp Gly Thr Phe Ile Arg Glu Lys Gly Lys Ser Val Arg
        115                 120                 125

Val His Val Gln Phe Phe Asp Asp Ser Pro Thr Arg Gly Trp Val Ser
    130                 135                 140

Lys Arg Leu Leu Lys Pro Tyr Thr Gly Ser Lys Ser Lys Glu Ala Gln
145                 150                 155                 160

Lys Gly Gly His Phe Tyr Ser Ala Lys Pro Glu Ile Leu Arg Ala Met
                165                 170                 175

Gln Arg Ala Asp Glu Ala Leu Asn Lys Asp Lys Ile Lys Arg Leu Glu
            180                 185                 190

Leu Ala Val Cys Asp Glu Pro Ser Glu Pro Glu Glu Glu Glu Glu Met
        195                 200                 205

Glu Val Gly Thr Thr Tyr Val Thr Asp Lys Ser Glu Glu Asp Asn Glu
    210                 215                 220

Ile Glu Ser Glu Glu Glu Val Gln Pro Lys Thr Gln Gly Ser Arg Arg
225                 230                 235                 240

Ser Ser Arg Gln Ile Lys Lys Arg Arg Val Ile Ser Asp Ser Glu Ser
                245                 250                 255

Asp Ile Gly Gly Ser Asp Val Glu Phe Lys Pro Asp Thr Lys Glu Glu
            260                 265                 270

Gly Ser Ser Asp Glu Ile Ser Ser Gly Val Gly Asp Ser Glu Ser Glu
        275                 280                 285

Gly Leu Asn Ser Pro Val Lys Val Ala Arg Lys Arg Lys Arg Met Val
    290                 295                 300
```

```
Thr Gly Asn Gly Ser Leu Lys Arg Lys Ser Ser Arg Lys Glu Thr Pro
305                 310                 315                 320

Ser Ala Thr Lys Gln Ala Thr Ser Ile Ser Ser Glu Thr Lys Asn Thr
                325                 330                 335

Leu Arg Ala Phe Ser Ala Pro Gln Asn Ser Glu Ser Gln Ala His Val
            340                 345                 350

Ser Gly Gly Gly Asp Asp Ser Ser Arg Pro Thr Val Trp Tyr His Glu
        355                 360                 365

Thr Leu Glu Trp Leu Lys Glu Glu Lys Arg Arg Asp Glu His Arg Arg
    370                 375                 380

Arg Pro Asp His Pro Asp Phe Asp Ala Ser Thr Leu Tyr Val Pro Glu
385                 390                 395                 400

Asp Phe Leu Asn Ser Cys Thr Pro Gly Met Arg Lys Trp Trp Gln Ile
                405                 410                 415

Lys Ser Gln Asn Phe Asp Leu Val Ile Cys Tyr Lys Val Gly Lys Phe
            420                 425                 430

Tyr Glu Leu Tyr His Met Asp Ala Leu Ile Gly Val Ser Glu Leu Gly
        435                 440                 445

Leu Val Phe Met Lys Gly Asn Trp Ala His Ser Gly Phe Pro Glu Ile
    450                 455                 460

Ala Phe Gly Arg Tyr Ser Asp Ser Leu Val Gln Lys Gly Tyr Lys Val
465                 470                 475                 480

Ala Arg Val Glu Gln Thr Glu Thr Pro Glu Met Met Glu Ala Arg Cys
                485                 490                 495

Arg Lys Met Ala His Ile Ser Lys Tyr Asp Arg Val Val Arg Arg Glu
            500                 505                 510

Ile Cys Arg Ile Ile Thr Lys Gly Thr Gln Thr Tyr Ser Val Leu Glu
        515                 520                 525

Gly Asp Pro Ser Glu Asn Tyr Ser Lys Tyr Leu Leu Ser Leu Lys Glu
    530                 535                 540

Lys Glu Glu Asp Ser Ser Gly His Thr Arg Ala Tyr Gly Val Cys Phe
545                 550                 555                 560
```

```
Val Asp Thr Ser Leu Gly Lys Phe Phe Ile Gly Gln Phe Ser Asp Asp
                565                 570                 575

Arg His Cys Ser Arg Phe Arg Thr Leu Val Ala His Tyr Pro Pro Val
            580                 585                 590

Gln Val Leu Phe Glu Lys Gly Asn Leu Ser Lys Glu Thr Lys Thr Ile
        595                 600                 605

Leu Lys Ser Ser Leu Ser Cys Ser Leu Gln Glu Gly Leu Ile Pro Gly
    610                 615                 620

Ser Gln Phe Trp Asp Ala Ser Lys Thr Leu Arg Thr Leu Leu Glu Glu
625                 630                 635                 640

Glu Tyr Phe Arg Glu Lys Leu Ser Asp Gly Ile Gly Val Met Leu Pro
                645                 650                 655

Gln Val Leu Lys Gly Met Thr Ser Glu Ser Asp Ser Ile Gly Leu Thr
            660                 665                 670

Pro Gly Glu Lys Ser Glu Leu Ala Leu Ser Ala Leu Gly Gly Cys Val
        675                 680                 685

Phe Tyr Leu Lys Lys Cys Leu Ile Asp Gln Glu Leu Leu Ser Met Ala
    690                 695                 700

Asn Phe Glu Glu Tyr Ile Pro Leu Asp Ser Asp Thr Val Ser Thr Thr
705                 710                 715                 720

Arg Ser Gly Ala Ile Phe Thr Lys Ala Tyr Gln Arg Met Val Leu Asp
                725                 730                 735

Ala Val Thr Leu Asn Asn Leu Glu Ile Phe Leu Asn Gly Thr Asn Gly
            740                 745                 750

Ser Thr Glu Gly Thr Leu Leu Glu Arg Val Asp Thr Cys His Thr Pro
        755                 760                 765

Phe Gly Lys Arg Leu Leu Lys Gln Trp Leu Cys Ala Pro Leu Cys Asn
    770                 775                 780

His Tyr Ala Ile Asn Asp Arg Leu Asp Ala Ile Glu Asp Leu Met Val
785                 790                 795                 800

Val Pro Asp Lys Ile Ser Glu Val Val Glu Leu Leu Lys Lys Leu Pro
                805                 810                 815
```

```
Asp Leu Glu Arg Leu Leu Ser Lys Ile His Asn Val Gly Ser Pro Leu
            820                 825                 830

Lys Ser Gln Asn His Pro Asp Ser Arg Ala Ile Met Tyr Glu Glu Thr
        835                 840                 845

Thr Tyr Ser Lys Lys Lys Ile Ile Asp Phe Leu Ser Ala Leu Glu Gly
    850                 855                 860

Phe Lys Val Met Cys Lys Ile Ile Gly Ile Met Glu Glu Val Ala Asp
865                 870                 875                 880

Gly Phe Lys Ser Lys Ile Leu Lys Gln Val Ile Ser Leu Gln Thr Lys
                885                 890                 895

Asn Pro Glu Gly Arg Phe Pro Asp Leu Thr Val Glu Leu Asn Arg Trp
            900                 905                 910

Asp Thr Ala Phe Asp His Glu Lys Ala Arg Lys Thr Gly Leu Ile Thr
        915                 920                 925

Pro Lys Ala Gly Phe Asp Ser Asp Tyr Asp Gln Ala Leu Ala Asp Ile
    930                 935                 940

Arg Glu Asn Glu Gln Ser Leu Leu Glu Tyr Leu Glu Lys Gln Arg Asn
945                 950                 955                 960

Arg Ile Gly Cys Arg Thr Ile Val Tyr Trp Gly Ile Gly Arg Asn Arg
                965                 970                 975

Tyr Gln Leu Glu Ile Pro Glu Asn Phe Thr Thr Arg Asn Leu Pro Glu
            980                 985                 990

Glu Tyr Glu Leu Lys Ser Thr Lys Lys Gly Cys Lys Arg Tyr Trp Thr
        995                1000                1005

Lys Thr Ile Glu Lys Lys Leu Ala Asn Leu Ile Asn Ala Glu Glu Arg
   1010                1015                1020

Arg Asp Val Ser Leu Lys Asp Cys Met Arg Arg Leu Phe Tyr Asn Phe
1025                1030                1035                1040

Asp Lys Asn Tyr Lys Asp Trp Gln Ser Ala Val Glu Cys Ile Ala Val
                1045                1050                1055

Leu Asp Val Leu Leu Cys Leu Ala Asn Tyr Ser Arg Gly Gly Asp Gly
           1060                1065                1070
```

Pro Met Cys Arg Pro Val Ile Leu Leu Pro Glu Asp Thr Pro Pro Phe
1075 1080 1085

Leu Glu Leu Lys Gly Ser Arg His Pro Cys Ile Thr Lys Thr Phe Phe
1090 1095 1100

Gly Asp Asp Phe Ile Pro Asn Asp Ile Leu Ile Gly Cys Glu Glu Glu
1105 1110 1115 1120

Glu Gln Glu Asn Gly Lys Ala Tyr Cys Val Leu Val Thr Gly Pro Asn
1125 1130 1135

Met Gly Gly Lys Ser Thr Leu Met Arg Gln Ala Gly Leu Leu Ala Val
1140 1145 1150

Met Ala Gln Met Gly Cys Tyr Val Pro Ala Glu Val Cys Arg Leu Thr
1155 1160 1165

Pro Ile Asp Arg Val Phe Thr Arg Leu Gly Ala Ser Asp Arg Ile Met
1170 1175 1180

Ser Gly Glu Ser Thr Phe Phe Val Glu Leu Ser Glu Thr Ala Ser Ile
1185 1190 1195 1200

Leu Met His Ala Thr Ala His Ser Leu Val Leu Val Asp Glu Leu Gly
1205 1210 1215

Arg Gly Thr Ala Thr Phe Asp Gly Thr Ala Ile Ala Asn Ala Val Val
1220 1225 1230

Lys Glu Leu Ala Glu Thr Ile Lys Cys Arg Thr Leu Phe Ser Thr His
1235 1240 1245

Tyr His Ser Leu Val Glu Asp Tyr Ser Gln Asn Val Ala Val Arg Leu
1250 1255 1260

Gly His Met Ala Cys Met Val Glu Asn Glu Cys Glu Asp Pro Ser Gln
1265 1270 1275 1280

Glu Thr Ile Thr Phe Leu Tyr Lys Phe Ile Lys Gly Ala Cys Pro Lys
1285 1290 1295

Ser Tyr Gly Phe Asn Ala Ala Arg Leu Ala Asn Leu Pro Glu Glu Val
1300 1305 1310

Ile Gln Lys Gly His Arg Lys Ala Arg Glu Phe Glu Lys Met Asn Gln
1315 1320 1325

Ser Leu Arg Leu Phe Arg Glu Val Cys Leu Ala Ser Glu Arg Ser Thr
1330 1335 1340

Val Asp Ala Glu Ala Val His Lys Leu Leu Thr Leu Ile Lys Glu Leu
1345 1350 1355 1360

<210> 27
<211> 4244
<212> DNA
<213> Homo sapiens

<400> 27
gccgcgcggt agatgcggtg cttttaggag ctccgtccga cagaacggtt gggccttgcc 60
ggctgtcggt atgtcgcgac agagcaccct gtacagcttc ttccccaagt ctccggcgct 120
gagtgatgcc aacaaggcct cggccagggc ctcacgcgaa ggcggccgtg ccgccgctgc 180
ccccggggcc tctccttccc caggcgggga tgcggcctgg agcgaggctg ggcctgggcc 240
caggcccttg gcgcgctccg cgtcaccgcc caaggcgaag aacctcaacg gagggctgcg 300
gagatcggta gcgcctgctg cccccaccag ttgtgacttc tcaccaggag atttggtttg 360
ggccaagatg gagggttacc cctggtggcc ttgtctggtt tacaaccacc cctttgatgg 420
aacattcatc cgcgagaaag ggaaatcagt ccgtgttcat gtacagtttt ttgatgacag 480
cccaacaagg ggctgggtta gcaaaaggct tttaaagcca tatacaggtt caaaatcaaa 540
ggaagcccag aagggaggtc atttttacag tgcaaagcct gaaatactga gagcaatgca 600
acgtgcagat gaagccttaa ataaagacaa gattaagagg cttgaattgg cagtttgtga 660
tgagccctca gagccagaag aggaagaaga gatggaggta ggcacaactt acgtaacaga 720
taagagtgaa gaagataatg aaattgagag tgaagaggaa gtacagccta agacacaagg 780
atctaggcga agtagccgcc aaataaaaaa acgaagggtc atatcagatt ctgagagtga 840
cattggtggc tctgatgtgg aatttaagcc agacactaag gaggaaggaa gcagtgatga 900
aataagcagt ggagtggggg atagtgagag tgaaggcctg aacagccctg tcaaagttgc 960
tcgaaagcgg aagagaatgg tgactggaaa tggctctctt aaaaggaaaa gctctaggaa 1020
ggaaacgccc tcagccacca aacaagcaac tagcatttca tcagaaacca agaatacttt 1080
gagagctttc tctgcccctc aaaattctga atcccaagcc cacgttagtg gaggtggtga 1140
tgacagtagt cgccctactg tttggtatca tgaaacttta gaatggctta aggaggaaaa 1200
gagaagagat gagcacagga ggaggcctga tcaccccgat tttgatgcat ctacactcta 1260
tgtgcctgag gatttcctca attcttgtac tcctgggatg aggaagtggt ggcagattaa 1320
gtctcagaac tttgatcttg tcatctgtta caaggtgggg aaatttttatg agctgtacca 1380
catggatgct cttattggag tcagtgaact ggggctggta ttcatgaaag gcaactgggc 1440
ccattctggc tttcctgaaa ttgcatttgg ccgttattca gattccctgg tgcagaaggg 1500
ctataaagta gcacgagtgg aacagactga gactccagaa atgatggagg cacgatgtag 1560
aaagatggca catatatcca agtatgatag agtggtgagg agggagatct gtaggatcat 1620
taccaagggt acacagactt acagtgtgct ggaaggtgat ccctctgaga actacagtaa 1680
gtatcttctt agcctcaaag aaaaagagga agattcttct ggccatactc gtgcatatgg 1740
tgtgtgcttt gttgatactt cactgggaaa gtttttcata ggtcagtttt cagatgatcg 1800
ccattgttcg agatttagga ctctagtggc acactatccc ccagtacaag ttttatttga 1860

aaaaggaaat ctctcaaagg aaactaaaac aattctaaag agttcattgt cctgttctct 1920
tcaggaaggt ctgatacccg gctcccagtt ttgggatgca tccaaaactt tgagaactct 1980
ccttgaggaa gaatatttta gggaaaagct aagtgatggc attggggtga tgttacccca 2040
ggtgcttaaa ggtatgactt cagagtctga ttccattggg ttgacaccag gagagaaaag 2100
tgaattggcc ctctctgctc taggtggttg tgtcttctac ctcaaaaaat gccttattga 2160
tcaggagctt ttatcaatgg ctaattttga agaatatatt cccttggatt ctgacacagt 2220
cagcactaca agatctggtg ctatcttcac caaagcctat caacgaatgg tgctagatgc 2280
agtgacatta aacaacttgg agatttttct gaatggaaca aatggttcta ctgaaggaac 2340
cctactagag agggttgata cttgccatac tccttttggt aagcggctcc taaagcaatg 2400
gctttgtgcc ccactctgta accattatgc tattaatgat cgtctagatg ccatagaaga 2460
cctcatggtt gtgcctgaca aaatctccga agttgtagag cttctaaaga agcttccaga 2520
tcttgagagg ctactcagta aaattcataa tgttgggtct cccctgaaga gtcagaacca 2580
cccagacagc agggctataa tgtatgaaga aactacatac agcaagaaga agattattga 2640
ttttctttct gctctggaag gattcaaagt aatgtgtaaa attataggga tcatggaaga 2700
agttgctgat ggttttaagt ctaaaatcct taagcaggtc atctctctgc agacaaaaaa 2760
tcctgaaggt cgttttcctg atttgactgt agaattgaac cgatgggata cagcctttga 2820
ccatgaaaag gctcgaaaga ctggacttat tactcccaaa gcaggctttg actctgatta 2880
tgaccaagct cttgctgaca taagagaaaa tgaacagagc ctcctggaat acctagagaa 2940
acagcgcaac agaattggct gtaggaccat agtctattgg gggattggta ggaaccgtta 3000
ccagctggaa attcctgaga atttcaccac tcgcaatttg ccagaagaat acgagttgaa 3060
atctaccaag aagggctgta aacgatactg gaccaaaact attgaaaaga agttggctaa 3120
tctcataaat gctgaagaac ggagggatgt atcattgaag gactgcatgc ggcgactgtt 3180
ctataacttt gataaaaatt acaaggactg gcagtctgct gtagagtgta tcgcagtgtt 3240
ggatgtttta ctgtgcctgg ctaactatag tcgagggggt gatggtccta tgtgtcgccc 3300
agtaattctg ttgccggaag ataccccccc cttcttagag cttaaaggat cacgccatcc 3360
ttgcattacg aagacttttt ttggagatga ttttattcct aatgacattc taataggctg 3420
tgaggaagag gagcaggaaa atggcaaagc ctattgtgtg cttgttactg gaccaaatat 3480
gggggggcaag tctacgctta tgagacaggc tggcttatta gctgtaatgg cccagatggg 3540
ttgttacgtc cctgctgaag tgtgcaggct cacaccaatt gatagagtgt ttactagact 3600
tggtgcctca gacagaataa tgtcaggtga aagtacattt tttgttgaat taagtgaaac 3660
tgccagcata ctcatgcatg caacagcaca ttctctggtg cttgtggatg aattaggaag 3720
aggtactgca acatttgatg ggacggcaat agcaaatgca gttgttaaag aacttgctga 3780
gactataaaa tgtcgtacat tattttcaac tcactaccat tcattagtag aagattattc 3840
tcaaaatgtt gctgtgcgcc taggacatat ggcatgcatg gtagaaaatg aatgtgaaga 3900
ccccagccag gagactatta cgttcctcta taaattcatt aagggagctt gtcctaaaag 3960
ctatggcttt aatgcagcaa ggcttgctaa tctcccagag gaagttattc aaaagggaca 4020
tagaaaagca agagaatttg agaagatgaa tcagtcacta cgattatttc gggaagtttg 4080
cctggctagt gaaaggtcaa ctgtagatgc tgaagctgtc cataaattgc tgactttgat 4140
taaggaatta tagactgact acattggaag ctttgagttg acttctgaca aaggtggtaa 4200
attcagacaa cattatgatc taataaactt tatttttaa aaat 4244

<210> 28
<211> 1128
<212> PRT
<213> Homo sapiens

<400> 28

```
Met Ser Arg Arg Lys Pro Ala Ser Gly Gly Leu Ala Ala Ser Ser Ser
 1               5                  10                  15

Ala Pro Ala Arg Gln Ala Val Leu Ser Arg Phe Phe Gln Ser Thr Gly
            20                  25                  30

Ser Leu Lys Ser Thr Ser Ser Ser Thr Gly Ala Ala Asp Gln Val Asp
        35                  40                  45

Pro Gly Ala Ala Ala Ala Ala Ala Pro Pro Ala Pro Ala Phe Pro Pro
    50                  55                  60

Gln Leu Pro Pro His Val Ala Thr Glu Ile Asp Arg Arg Lys Lys Arg
65                  70                  75                  80

Pro Leu Glu Asn Asp Gly Pro Val Lys Lys Lys Val Lys Lys Val Gln
                85                  90                  95

Gln Lys Glu Gly Gly Ser Asp Leu Gly Met Ser Gly Asn Ser Glu Pro
            100                 105                 110

Lys Lys Cys Leu Arg Thr Arg Asn Val Ser Lys Ser Leu Glu Lys Leu
        115                 120                 125

Lys Glu Phe Cys Cys Asp Ser Ala Leu Pro Gln Ser Arg Val Gln Thr
    130                 135                 140

Glu Ser Leu Gln Glu Arg Phe Ala Val Leu Pro Lys Cys Thr Asp Phe
145                 150                 155                 160

Asp Asp Ile Ser Leu Leu His Ala Lys Asn Ala Val Ser Ser Glu Asp
                165                 170                 175

Ser Lys Arg Gln Ile Asn Gln Lys Asp Thr Thr Leu Phe Asp Leu Ser
            180                 185                 190

Gln Phe Gly Ser Ser Asn Thr Ser His Glu Asn Leu Gln Lys Thr Ala
        195                 200                 205

Ser Lys Ser Ala Asn Lys Arg Ser Lys Ser Ile Tyr Thr Pro Leu Glu
    210                 215                 220

Leu Gln Tyr Ile Glu Met Lys Gln Gln His Lys Asp Ala Val Leu Cys
225                 230                 235                 240

Val Glu Cys Gly Tyr Lys Tyr Arg Phe Phe Gly Glu Asp Ala Glu Ile
                245                 250                 255
```

```
Ala Ala Arg Glu Leu Asn Ile Tyr Cys His Leu Asp His Asn Phe Met
            260                 265                 270

Thr Ala Ser Ile Pro Thr His Arg Leu Phe Val His Val Arg Arg Leu
        275                 280                 285

Val Ala Lys Gly Tyr Lys Val Gly Val Val Lys Gln Thr Glu Thr Ala
    290                 295                 300

Ala Leu Lys Ala Ile Gly Asp Asn Arg Ser Ser Leu Phe Ser Arg Lys
305                 310                 315                 320

Leu Thr Ala Leu Tyr Thr Lys Ser Thr Leu Ile Gly Glu Asp Val Asn
                325                 330                 335

Pro Leu Ile Lys Leu Asp Asp Ala Val Asn Val Asp Glu Ile Met Thr
            340                 345                 350

Asp Thr Ser Thr Ser Tyr Leu Leu Cys Ile Ser Glu Asn Lys Glu Asn
        355                 360                 365

Val Arg Asp Lys Lys Lys Gly Asn Ile Phe Ile Gly Ile Val Gly Val
    370                 375                 380

Gln Pro Ala Thr Gly Glu Val Val Phe Asp Ser Phe Gln Asp Ser Ala
385                 390                 395                 400

Ser Arg Ser Glu Leu Glu Thr Arg Met Ser Ser Leu Gln Pro Val Glu
                405                 410                 415

Leu Leu Leu Pro Ser Ala Leu Ser Glu Gln Thr Glu Ala Leu Ile His
            420                 425                 430

Arg Ala Thr Ser Val Ser Val Gln Asp Asp Arg Ile Arg Val Glu Arg
        435                 440                 445

Met Asp Asn Ile Tyr Phe Glu Tyr Ser His Ala Phe Gln Ala Val Thr
    450                 455                 460

Glu Phe Tyr Ala Lys Asp Thr Val Asp Ile Lys Gly Ser Gln Ile Ile
465                 470                 475                 480

Ser Gly Ile Val Asn Leu Glu Lys Pro Val Ile Cys Ser Leu Ala Ala
                485                 490                 495

Ile Ile Lys Tyr Leu Lys Glu Phe Asn Leu Glu Lys Met Leu Ser Lys
            500                 505                 510
```

```
Pro Glu Asn Phe Lys Gln Leu Ser Ser Lys Met Glu Phe Met Thr Ile
        515                 520                 525

Asn Gly Thr Thr Leu Arg Asn Leu Glu Ile Leu Gln Asn Gln Thr Asp
    530                 535                 540

Met Lys Thr Lys Gly Ser Leu Leu Trp Val Leu Asp His Thr Lys Thr
545                 550                 555                 560

Ser Phe Gly Arg Arg Lys Leu Lys Lys Trp Val Thr Gln Pro Leu Leu
                565                 570                 575

Lys Leu Arg Glu Ile Asn Ala Arg Leu Asp Ala Val Ser Glu Val Leu
            580                 585                 590

His Ser Glu Ser Ser Val Phe Gly Gln Ile Glu Asn His Leu Arg Lys
        595                 600                 605

Leu Pro Asp Ile Glu Arg Gly Leu Cys Ser Ile Tyr His Lys Lys Cys
    610                 615                 620

Ser Thr Gln Glu Phe Phe Leu Ile Val Lys Thr Leu Tyr His Leu Lys
625                 630                 635                 640

Ser Glu Phe Gln Ala Ile Ile Pro Ala Val Asn Ser His Ile Gln Ser
                645                 650                 655

Asp Leu Leu Arg Thr Val Ile Leu Glu Ile Pro Glu Leu Leu Ser Pro
            660                 665                 670

Val Glu His Tyr Leu Lys Ile Leu Asn Glu Gln Ala Ala Lys Val Gly
        675                 680                 685

Asp Lys Thr Glu Leu Phe Lys Asp Leu Ser Asp Phe Pro Leu Ile Lys
    690                 695                 700

Lys Arg Lys Asp Glu Ile Gln Gly Val Ile Asp Glu Ile Arg Met His
705                 710                 715                 720

Leu Gln Glu Ile Arg Lys Ile Leu Lys Asn Pro Ser Ala Gln Tyr Val
                725                 730                 735

Thr Val Ser Gly Gln Glu Phe Met Ile Glu Ile Lys Asn Ser Ala Val
            740                 745                 750

Ser Cys Ile Pro Thr Asp Trp Val Lys Val Gly Ser Thr Lys Ala Val
        755                 760                 765
```

```
Ser Arg Phe His Ser Pro Phe Ile Val Glu Asn Tyr Arg His Leu Asn
    770                 775                 780

Gln Leu Arg Glu Gln Leu Val Leu Asp Cys Ser Ala Glu Trp Leu Asp
785                 790                 795                 800

Phe Leu Glu Lys Phe Ser Glu His Tyr His Ser Leu Cys Lys Ala Val
                805                 810                 815

His His Leu Ala Thr Val Asp Cys Ile Phe Ser Leu Ala Lys Val Ala
            820                 825                 830

Lys Gln Gly Asp Tyr Cys Arg Pro Thr Val Gln Glu Glu Arg Lys Ile
        835                 840                 845

Val Ile Lys Asn Gly Arg His Pro Val Ile Asp Val Leu Leu Gly Glu
    850                 855                 860

Gln Asp Gln Tyr Val Pro Asn Asn Thr Asp Leu Ser Glu Asp Ser Glu
865                 870                 875                 880

Arg Val Met Ile Ile Thr Gly Pro Asn Met Gly Gly Lys Ser Ser Tyr
                885                 890                 895

Ile Lys Gln Val Ala Leu Ile Thr Ile Met Ala Gln Ile Gly Ser Tyr
            900                 905                 910

Val Pro Ala Glu Glu Ala Thr Ile Gly Ile Val Asp Gly Ile Phe Thr
        915                 920                 925

Arg Met Gly Ala Ala Asp Asn Ile Tyr Lys Gly Arg Ser Thr Phe Met
    930                 935                 940

Glu Glu Leu Thr Asp Thr Ala Glu Ile Ile Arg Lys Ala Thr Ser Gln
945                 950                 955                 960

Ser Leu Val Ile Leu Asp Glu Leu Gly Arg Gly Thr Ser Thr His Asp
                965                 970                 975

Gly Ile Ala Ile Ala Tyr Ala Thr Leu Glu Tyr Phe Ile Arg Asp Val
            980                 985                 990

Lys Ser Leu Thr Leu Phe Val Thr His Tyr Pro Pro Val Cys Glu Leu
        995                1000                1005

Glu Lys Asn Tyr Ser His Gln Val Gly Asn Tyr His Met Gly Phe Leu
   1010                1015                1020
```

Val Ser Glu Asp Glu Ser Lys Leu Asp Pro Gly Ala Ala Glu Gln Val
1025 1030 1035 1040

Pro Asp Phe Val Thr Phe Leu Tyr Gln Ile Thr Arg Gly Ile Ala Ala
1045 1050 1055

Arg Ser Tyr Gly Leu Asn Val Ala Lys Leu Ala Asp Val Pro Gly Glu
1060 1065 1070

Ile Leu Lys Lys Ala Ala His Lys Ser Lys Glu Leu Glu Gly Leu Ile
1075 1080 1085

Asn Thr Lys Arg Lys Arg Leu Lys Tyr Phe Ala Lys Leu Trp Thr Met
1090 1095 1100

His Asn Ala Gln Asp Leu Gln Lys Trp Thr Glu Glu Phe Asn Met Glu
1105 1110 1115 1120

Glu Thr Gln Thr Ser Leu Leu His
1125

<210> 29
<211> 4374
<212> DNA
<213> Homo sapiens

<400> 29
gggcacgagc cctgccatgt ctcgccggaa gcctgcgtcg ggcggcctcg ctgcctccag 60
ctcagccccc gcgaggcaag cggttttgag ccgattcttc cagtctacgg gaagcctgaa 120
atccacctcc tcctccacag gtgcagccga ccaggtggac cctggcgctg cagcggccgc 180
agcgccccca gcgcccgcct tcccgcccca gctgccgccg cacgtagcta cagaaattga 240
cagaagaaag aagagaccat tggaaaatga tgggcctgtt aaaaagaaag taaagaaagt 300
ccaacaaaag gaaggaggaa gtgatctggg aatgtctggc aactctgagc caaagaaatg 360
tctgaggacc aggaatgttt caaagtctct ggaaaaattg aaagaattct gctgcgattc 420
tgcccttcct caaagtagag tccagacaga atctctgcag gagagatttg cagttctgcc 480
aaaatgtact gattttgatg atatcagtct tctacacgca aagaatgcag tttcttctga 540
agattcgaaa cgtcaaatta atcaaaagga cacaacactt tttgatctca gtcagtttgg 600
atcatcaaat acaagtcatg aaaatttaca gaaaactgct tccaaatcag ctaacaaacg 660
gtccaaaagc atctatacgc cgctagaatt acaatacata gaaatgaagc agcagcacaa 720
agatgcagtt ttgtgtgtgg aatgtggata taagtataga ttctttgggg aagatgcaga 780
gattgcagcc cgagagctca atatttattg ccatttagat cacaacttta tgacagcaag 840
tatacctact cacagactgt ttgttcatgt acgccgcctg gtggcaaaag gatataaggt 900
gggagttgtg aagcaaactg aaactgcagc attaaaggcc attggagaca acagaagttc 960
actcttttcc cggaaattga ctgcccttta tacaaaatct acacttattg gagaagatgt 1020
gaatccccta atcaagctgg atgatgctgt aaatgttgat gagataatga ctgatacttc 1080
taccagctat cttctgtgca tctctgaaaa taaggaaaat gttagggaca aaaaaaaggg 1140
caacattttt attggcattg tgggagtgca gcctgccaca ggcgaggttg tgtttgatag 1200

tttccaggac tctgcttctc gttcagagct agaaacccgg atgtcaagcc tgcagccagt 1260
agagctgctg cttccttcgg ccttgtccga gcaaacagag gcgctcatcc acagagccac 1320
atctgttagt gtgcaggatg acagaattcg agtcgaaagg atggataaca tttattttga 1380
atacagccat gctttccagg cagttacaga gttttatgca aaagatacag ttgacatcaa 1440
aggttctcaa attatttctg gcattgttaa cttagagaag cctgtgattt gctctttggc 1500
tgccatcata aaatacctca aagaattcaa cttggaaaag atgctctcca aacctgagaa 1560
ttttaaacag ctatcaagta aaatggaatt tatgacaatt aatggaacaa cattaaggaa 1620
tctggaaatc ctacagaatc agactgatat gaaaaccaaa ggaagtttgc tgtgggtttt 1680
agaccacact aaaacttcat ttgggagacg gaagttaaag aagtgggtga cccagccact 1740
ccttaaatta agggaaataa atgcccggct tgatgctgta tcggaagttc tccattcaga 1800
atctagtgtg tttggtcaga tagaaaatca tctacgtaaa ttgcccgaca tagagagggg 1860
actctgtagc atttatcaca aaaaatgttc tacccaagag ttcttcttga ttgtcaaaac 1920
tttatatcac ctaaagtcag aatttcaagc aataatacct gctgttaatt cccacattca 1980
gtcagacttg ctccggaccg ttattttaga aattcctgaa ctcctcagtc cagtggagca 2040
ttacttaaag atactcaatg aacaagctgc caaagttggg gataaaactg aattatttaa 2100
agacctttct gacttccctt taataaaaaa gaggaaggat gaaattcaag gtgttattga 2160
cgagatccga atgcatttgc aagaaatacg aaaaatacta aaaaatcctt ctgcacaata 2220
tgtgacagta tcaggacagg agtttatgat agaaataaag aactctgctg tatcttgtat 2280
accaactgat tgggtaaagg ttggaagcac aaaagctgtg agccgctttc actctccttt 2340
tattgtagaa aattacagac atctgaatca gctccgggag cagctagtcc ttgactgcag 2400
tgctgaatgg cttgattttc tagagaaatt cagtgaacat tatcactcct tgtgtaaagc 2460
agtgcatcac ctagcaactg ttgactgcat tttctccctg gccaaggtcg ctaagcaagg 2520
agattactgc agaccaactg tacaagaaga aagaaaaatt gtaataaaaa atggaaggca 2580
ccctgtgatt gatgtgttgc tgggagaaca ggatcaatat gtcccaaata atacagattt 2640
atcagaggac tcagagagag taatgataat taccggacca aacatgggtg gaaagagctc 2700
ctacataaaa caagttgcat tgattaccat catggctcag attggctcct atgttcctgc 2760
agaagaagcg acaattggga ttgtggatgg cattttcaca aggatgggtg ctgcagacaa 2820
tatatataaa ggacggagta catttatgga agaactgact gacacagcag aaataatcag 2880
aaaagcaaca tcacagtcct tggttatctt ggatgaacta ggaagaggga cgagcactca 2940
tgatggaatt gccattgcct atgctacact tgagtatttc atcagagatg tgaaatcctt 3000
aaccctgttt gtcacccatt atccgccagt ttgtgaacta gaaaaaaatt actcacacca 3060
ggtggggaat taccacatgg gattcttggt cagtgaggat gaaagcaaac tggatccagg 3120
cgcagcagaa caagtccctg attttgtcac cttcctttac caaataacta gaggaattgc 3180
agcaaggagt tatggattaa atgtggctaa actagcagat gttcctggag aaattttgaa 3240
gaaagcagct cacaagtcaa aagagctgga aggattaata aatacgaaaa gaaagagact 3300
caagtatttt gcaaagttat ggacgatgca taatgcacaa gacctgcaga agtggacaga 3360
ggagttcaac atggaagaaa cacagacttc tcttcttcat taaaatgaag actacatttg 3420
tgaacaaaaa atggagaatt aaaaatacca actgtacaaa ataactctcc agtaacagcc 3480
tatctttgtg tgacatgtga gcataaaatt atgaccatgg tatattccta ttggaaacag 3540
agaggttttt ctgaagacag tctttttcaa gtttctgtct tcctaacttt tctacgtata 3600
aacactcttg aatagacttc cactttgtaa ttagaaaatt ttatggacag taagtccagt 3660
aaagccttaa gtggcagaat ataattccca agcttttgga gggtgatata aaaatttact 3720
tgatattttt atttgtttca gttcagataa ttggcaactg ggtgaatctg gcaggaatct 3780
atccattgaa ctaaaataat tttattatgc aaccagttta tccaccaaga acataagaat 3840
tttttataag tagaaagaat tggccaggca tggtggctca tgcctgtaat cccagcactt 3900
tgggaggcca aggtaggcag atcacctgag gtcaggagtt caagaccagc ctggccaaca 3960
tggcaaaacc ccatctttac taaaaatata aagtacatct ctactaaaaa tacgaaaaaa 4020
ttagctgggc atggtggcgc acacctgtag tcccagctac tccggaggct gaggcaggag 4080

aatctcttga acctgggagg cggaggttgc aatgagccga gatcacgtca ctgcactcca 4140
gcttgggcaa cagagcaaga ctccatctca aaaaagaaaa aagaaaagaa atagaattat 4200
caagctttta aaaactagag cacagaagga ataaggtcat gaaatttaaa aggttaaata 4260
ttgtcatagg attaagcagt ttaaagattg ttggatgaaa ttatttgtca ttcattcaag 4320
taataaatat ttaatgaata cttgctataa aaaaaaaaaa aaaaaaaaaa aaaa 4374

<210> 30
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 30
gatatctcca ctgacgtaag 20

<210> 31
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 31
tgttgccggt cttgcgatg 19

<210> 32
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 32
cccgatctag taacatagat g 21

<210> 33
<211> 21

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 33
cagtctggat cgcgaaaact g 21

<210> 34
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 34
ggtgattacc gacgaaaacg 20

<210> 35
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 35
agtgaagggc gaacagttcc 20

<210> 36
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 36
gagtattgcc aacgaacc 18

<210> 37
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 37
gtatcaccgc gtctttgatc 20

<210> 38
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 38
cgaaacgcag cacgatacg 19

<210> 39
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 39
gttcaacgct gacatcacc 19

<210> 40
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide

primer

<400> 40
catgttcatc tgcccagtcg 20

<210> 41
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 41
gctttggaca taccatcc 18

<210> 42
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 42
caccgaagtt catgccag 18

<210> 43
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 43
tgactacttt tgacttcagc c 21

<210> 44
<211> 22
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide primer

<400> 44
aaccattcaa catttttaac cc 22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/00934

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A01H 1/06; A61K 31/7076, 31/7088; C12N 1/00, 5/00; C12Q 1/68
US CL : 435/6, 375, 410; 514/44; 536/24.5; 800/13, 276, 286, 295

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/6, 375, 410; 514/44; 536/24.5; 800/13, 276, 286, 295

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
USPAT, JPABS, EPABS, DWPI, BIOSIS, CAPLUS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	US 6,191,268 B1 (LISKAY et al.) 20 February 2001.	1-71
X	US 6,146,894 A (NICOLAIDES et al.) 14 November 2000, see entire document.	23-53, 71
X	US 5,907,079 A (MAK et al.) 25 May 1999, see entire document.	1,17-20, 23-27, 29, 31-34, 36-39
X	WO 99/19492 A2 (RHONE-POULENC AGRO) 22 April 1999, see pages 4-33.	1, 17-19, 23, 24, 26, 29-31, 41-49, 52
X	YU et al. Adriamycin Induces Large Deletions in a Major Type of Mutation in CHO Cells. Mutation Research. November 1994, Vol. 325, Nos. 2-3, pages 91-98, see entire document.	1-3, 20, 23, 24, 26-29, 31, 56, 57
X	CHAKRAVARTI et al. Relating Aromatic Hydrocarbon-Induced DNA Adducts and c-H-ras Mutations in Mouse Skin papillomas: the Role of Apurinic Sites. Proceedings of the national Academy of Sciences, USA. October 1995, Vol. 92, Pages 10422-10426. See entire document including Figure 1.	1-6, 23, 24, 26-29, 31-34 36-38, 56-60
X	DRUMMOND et al. Cisplatin and Adriamycin Resistance are Associated with MutLa and Mismatch Repair Deficiency in an Ovarian Cell line. The Journal of Biological Chemistry. 16 August 1996, Vol. 271, No. 33, pages 19645-19648, see entire document.	1-3, 20, 21, 23, 24, 26-29, 31, 49, 51, 56, 57, 71

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
16 March 2001 (16.03.2001)

Date of mailing of the international search report
26 APR 2001

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703)305-3230

Authorized officer
Thomas Larson

Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/00934

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	QUIAN et al. Molecular Events after Antisense inhibition of hMSH2 in a HeLa Cell Line. Mutation Research. 12 October 1998, Vol. 418, Nos. 2-3, pages 61-71, see entire document.	1, 17, 23-31